UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

BACKGROUND MORTALITY OF SAIGA ANTELOPE (*Saiga t. tatarica*) DURING CALVING SEASON IN KAZAKHSTAN

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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ABSTRACT

BACKGROUND MORTALITY OF SAIGA ANTELOPE (*Saiga t. tatarica*) DURING CALVING SEASON IN KAZAKHSTAN

The temporary calving aggregation of the endangered Saiga antelope (*Saiga tatarica*) was monitored in the Betpak-Dala population, Amangeldy region, Kostanay oblast, in Kazakhstan, aiming to quantify and characterize regular patterns of mortality at this critical time for the species. Animals were located through GPS-guided linear transects walked across the calving area within a period of 10 days. Animal carcasses were necropsied in the field to investigate proximate causes of death.

An average density of 0.1 (95%, CI 0.07 - 0.12) calf carcasses per hectare was measured in the surveyed area. Thus, a total of 1314 carcasses (95%, CI 920 - 1577) was estimated for the whole calving area. The mortality rate of calves during the calving period was estimated at 4.4% (95%, CI 2.3 - 6.6). Although these estimates and respective CI's were calculated, they possibly underrepresent mortality in the population. Higher densities of dead calves found later in the study likely reflected changing weather conditions leading to increased mortality. The study also reveals sex differences in saiga newborn mortality, with dead females more often recorded (ratio 2:1). This might be related to lower birth mass of females compared to males. In 47 newborn calves examined post-mortem, the main presumptive causes of death were nutritional and/or climatic stress (34%), dystocia (23.4%) and stillbirths (12.8%). The findings suggest that predation was a minor source of calf mortality, apparently due to a low abundance of predators in the area.

In adult saiga, mortality was negligible, as indicated by a low average density of animals in the surveyed area, measured at 0.005 (95%, CI 0.003 - 0.007) carcasses per hectare. A total of 70 (95%, CI 46 - 94) adult carcasses was estimated for the entire calving area. Adult mortality involved only females. Reproductive disorders, including dystocia and uterine prolapse, were the main cause of mortality observed, occurring in 17 (70.8%) of 24 females examined.

The mortality baseline information generated by this study proves useful to amend the monitoring efficacy of future calving seasons, as well as to improve early detection of events leading to unusual mortality patterns in Saiga antelope during this period.

Key words: *Saiga tatarica*, mortality, calving season, Betpak-Dala population, Kazakhstan

MORTALIDADE-BASE DO ANTÍLOPE SAIGA (*Saiga t. tatarica*)
DURANTE A ÉPOCA DE PARIÇÃO NO CAZAQUISTÃO

A agregação temporária na época de partos do antílope Saiga (*Saiga tatarica*) foi monitorizada em 2014 na população Betpak-Dala, região Amangeldy, província de Kostanay, no Cazaquistão, com o objetivo de quantificar e caracterizar a mortalidade de base durante este período crítico para a sobrevivência desta espécie ameaçada de extinção. Os animais foram localizados recorrendo a transectos lineares guiados por GPS, percorridos a pé, atravessando as áreas de parto, durante um período de 10 dias. Os animais mortos foram necropsiados no campo para identificar as causas mais prováveis de morte.
A densidade média de neonatos mortos na área pesquisada foi de 0,1 cadáveres por hectare (95% IC 0,07 - 0,12). O total de mortes de neonatos em toda a área de partos foi estimado em 1314 (95% IC 920 - 1577). A taxa de mortalidade em bezerros foi estimada em 4,4% (95% IC 2.3 - 6.6). Embora estas estimativas, com respectivos IC's, tenham sido calculadas, as mesmas são susceptíveis de sub-representar a mortalidade na população. Densidades mais altas de bezerros mortos observadas no final do período de estudo refletiram provavelmente alterações nas condições climáticas e consequente aumento da mortalidade O estudo revelou ainda uma frequência de mortalidade superior em fêmeas (rácio 2:1). Isto poderá estar relacionado com pesos ao nascimento inferiores aos dos machos. Em 47 bezerros examinados pós-mortem, as principais causas presuntivas de morte foram stress nutricional e/ou climático (34%), distócia (23,4%) e nados-mortos (12,8%). Os resultados sugerem que a predação foi uma causa pouco significativa de mortalidade neonatal, aparentemente devido a uma baixa abundância de predadores na área.
Em animais adultos, a mortalidade observada durante este período foi negligenciável. A densidade média de adultos mortos na área pesquisada foi de 0,005 (95%, IC de 0,003 - 0,007) cadáveres por hectare. estimando-se, assim, um total de 70 (95%, IC 46, 94) cadáveres adultos em toda a área de agregação. A mortalidade observada atingiu apenas fêmeas. Os distúrbios reprodutivos, incluindo distócia e prolapso uterino, constituíram a principal causa de mortalidade, ocorrendo em 17 (70,8%) de 24 fêmeas examinadas.
A informação sobre mortalidade de base gerada por este estudo é útil para melhorar a monitorização de futuras épocas de parto bem como a detecção precoce de eventos que conduzam a padrões de mortalidade incomuns no antílope Saiga durante este periodo.

Palavras-chave: *Saiga tatarica*, mortalidade, época de parição, população Betpak-Dala, Cazaquistão.

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ACRONYMS AND ABBREVIATIONS

ACBK - Association for the Conservation of the Biodiversity of Kazakhstan

CFW - Committee of Forestry and Wildlife of the Ministry of Agriculture of the Republic of Kazakhstan

CMS - Convention on the Conservation of Migratory Species of Wild Animals

CI - Confidence interval

FAO - The Food and Agriculture Organization of the United Nations

FMD - Foot and Mouth Disease

FMV-UL - Faculty of Veterinary Medicine, University of Lisbon

GAM - Generalized Additive Model

GPS - Global Positioning System

IUCN - International Union for Conservation of Nature

LOWESS - Locally Weighted Scatterplot Smoothing

OIE - World Organisation for Animal Health

PMNs - Polymorphnuclear neutrophils

RVC - Royal Veterinary College, University of London

SD - Standard deviation

SE - Standard error of the mean

SOP - Standard operating procedures

RIBSP - Research Institute for Biological Safety Problems

1 THEORETICAL INTRODUCTION

1.1 DESCRIPTION OF THE SPECIES

The Saiga (*Saiga tatarica*, Bovidae, Mammalia; Nowak, 1999), found in the semi-desert and steppe regions of Central Asia, is a medium-sized ungulate, sandy in colour and very distinctive in its appearance, with a protuberant nose that is thought to be an adaptation to its extremely cold and dusty environment, also serving during the mating season as a weapon of intimidation against rival males and a point of attraction of females in heat (Bekenov, Grachev, & Milner-Gulland, 1998; Frey, Volodin, & Volodina, 2007). Slightly larger and heavier than their female counterparts, males also possess unusual, semi-translucent horns (Fadeev & Sludskiy, 1982).

Figure 1 - Adult male saiga in winter in Tengiz region of Kazakhstan (Photograph © Klaus Nigge. Source: http://www.geo.ru).

There are two distinct sub-species of Saiga antelope: *Saiga tatarica tatarica*, in Kazakhstan, Russia, Uzbekistan and Turkmenistan; and *Saiga tatarica mongolica* in Mongolia. *Saiga t. tatarica*, on which this study focuses, undertakes large-scale seasonal migrations between its summer and winter ranges, due to the extreme variation in climate conditions and to its need for new pastures. Migration characteristics, such as periods and distances, differ from year to year, depending on environmental conditions like climate, quality of pastures and water accessibility, obstacles across migration routes and disturbances experienced by the herd (Bekenov et al., 1998). Saiga generally migrates north or northwest in early spring, with a brief halt to congregate for calving, and south or southeast in autumn (Bekenov, Blank, Grachev, & Plakhov, 2001). The calving sites, selected by the animals during spring migration (March-May), are also highly variable from year to year depending not only on environmental factors but also on human disturbance (Bekenov et al., 1998; Singh, Grachev, & Milner-Gulland, 2010). A significant change in calving locations was observed in the 2000s, with the areas occurring further north and away from human settlements than in previous decades (Singh et al., 2010).

Saiga are gregarious animals, with extremely fluid herd structures, exception made for family groups of females with calves, which are more stable. Groups vary from tens to thousands at certain times: during calving and migration (Fadeev & Sludskiy, 1982; Bekenov et al., 1998).

Figure 2 - A group of adult females saiga and their young migrating to summer ranges in Kazakhstan (Courtesy of Albert Salemgareyev, ACBK).

Saiga have a harem breeding system, where dominant males form and defend groups of up to 15 (sometimes 30) females of different ages (Fadeev & Sludskiy, 1982; Bekenov et al., 1998). These harems begin to form around mid-November and mating commonly takes place on large scale within a period of about 10 days in December, sometimes early January, depending on the population (Bekenov et al., 1998). Saiga are recognized as having a high reproductive potential (Fadeev & Sludskiy, 1982). Females reach sexual maturity at the age of 7-8 months, most of them producing young in the first year if climatic conditions are favourable and food sufficiently available during their growth. Males, in turn, become sexually mature around 19 months of age and usually have a shorter life, presumably as a result of high natural mortality among males (e.g. due to fighting during the rut) and selective hunting for horns (Fadeev & Sludskiy, 1982; Bekenov et al., 1998). Fertility rates recorded over the period of 1964-1978 in Kazakhstan were on average 92.3% (range 68.2-100, n=300) in yearling females and 98.3% (range 90.8-100, n=592) in older females (Fadeev & Sludskiy, 1982). Twinning rates are high among older females (Fadeev & Sludskiy, 1982; Bekenov et al., 1998; Kühl et al., 2009[b]). Overall twinning rate recorded among ≥ 2 years old females was 79.4% in the period between 1964-1978 (range 57.2-92, n=592), but averaging only around 4% in yearlings (range 4-11.9%, n=300; Fadeev & Sludskiy, 1982). Triplets may also occur, although this is a far rarer event. Given that females mature so early and often produce twins after their first calving year, saiga are able to expand their population quickly if environmental conditions permit it. Changes in fertility and fecundity are recorded, however, as a result of unfavourable climatic conditions (Fadeev & Sludskiy, 1982; Coulson, Milner-Gulland, & Clutton-Brock, 2000) or disturbance during the breeding season (Bekenov et al*.,* 1998). Significant decreases in sexually mature males in the saiga populations, mainly due to selective overhunting, also have a negative impact on saiga productivity (Milner-Gulland et al., 2003; Kühl et al*.,* 2009[b]), as observed in the Kalmykia population in Russia, in which the fecundity levels dropped considerably in 2001, when a proportion of less than 2.5% adult males during the rut was recorded (Milner-Gulland et al. 2003). Moreover, the twinning rates for this population in the period between 2003 and 2006 were 20.9% lower than those recorded in 1995-2001(Kühl, 2008; Kühl et al*.,* 2009[b]).

The birth season for Saiga antelopes lasts from late April to early June, with mass calving commonly taking place within a short period of 5-8 days (Fadeev & Sludskiy, 1982; Bekenov et al., 1998; Kühl, 2008). Calving seems to be scheduled to coincide with a period of intensive growth of vegetation and more favorable weather conditions for calf survival (Zhirnov, Bekenov, Grachev, & Pronyaev, 1998; Bekenov et al., 1998; Singh et al., 2010). At this time, typically tens of thousands of animals, mostly females, congregate in areas where there is good pasture, watering places and minimal disturbance (Bekenov et al., 1998; Singh et al., 2010).

Figure 3 - Main 2014 calving aggregation of the Betpak-Dala saiga population in Kazakhstan.

In the period of 1965-1993, birth aggregations ranging from 50,000 to 150,000 animals (maximum 200,000) in areas of 150-900 km^2, sometimes 9000 km^2, were commonly observed in Kazakhstan. A single population may form more than one of these large birth aggregations (Fadeev & Sludskiy 1982; Bekenov et al., 1998). This spatial clustering of births is assumed to be a mechanism of predator avoidance, particularly wolves, which can be a major cause of calf mortality (Fadeev & Sludskiy 1982; Bekenov et al., 1998; Milner-Gulland, 2001). Saiga displays a typical hiding behaviour, i.e., newborn calves remain hidden in the birth places for the first 2 days of life, and at the age of 4-5 days they are able to follow their mothers (Bannikov, 1963; Bekenov et al., 1998).

Figure 4 - Newborn saiga lying motionless in the birth areas, Torgai region of Kazakhstan (Courtesy of Dr. Richard Kock, RVC).

1.2 CURRENT STATUS AND CONSERVATION IN KAZAKHSTAN

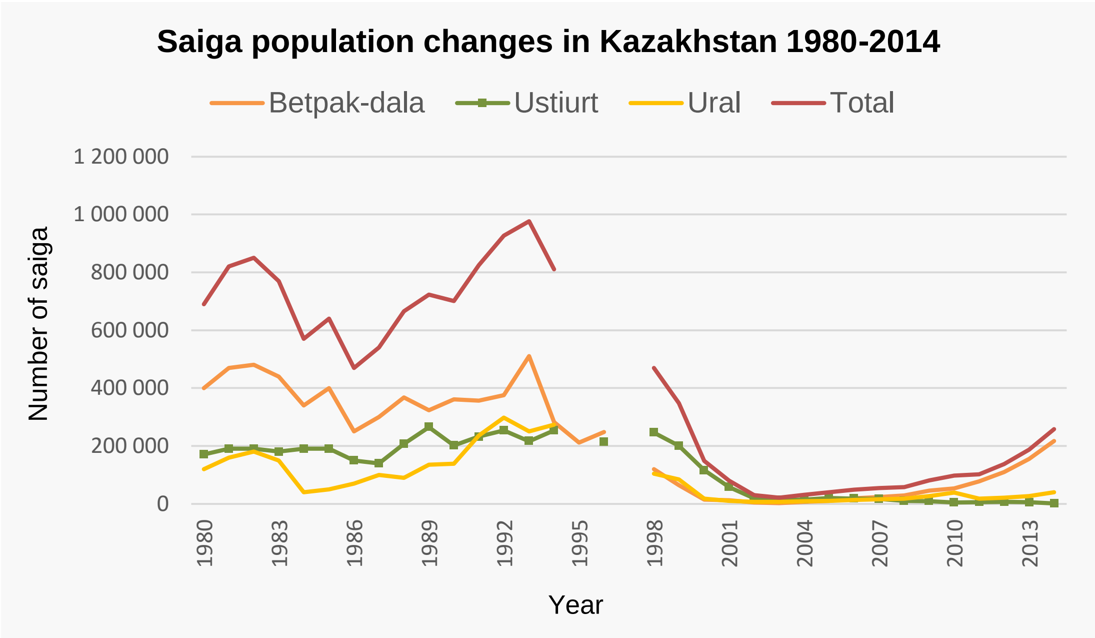
Saiga antelope currently occurs in five widely distributed populations: Western Mongolia (*Saiga . t. mongolica*), Northwest pre-Caspian in Russia, and Ural, Ustiurt and Betpak-Dala in Kazakhstan. A sixth population of *Saiga t. tatarica*, once present in Northwest China and adjacent regions of Mongolia, became extinct in the 1960s (CMS, 2006).

The Ural population is found in the far west of Kazakhstan, between the Volga and Ural rivers, overlapping with Russia in a small extent (Figure 5). The Ustiurt population occurs between the Caspian and Aral Seas, and is also a transboundary population, moving to Uzbekistan in winter and rarely to Northern Turkmenistan in harsh winters. The Betpak-Dala population, on which this study focuses, is distributed over a large area to the north and east of the Aral Sea in Central Kazakhstan (Bekenov et al., 1998; Gorelov, 2001; Marmazinskaya & Mardanov, 2001; CMS, 2006). Based on telemetry data and field monitoring, saiga are sometimes found further southeast of the Betpak-Dala population's range, but in small numbers, probably due to higher poaching or disturbance in the area (S. Zuther, pers. communication, 2014).

Figure 5 - The current distribution of Saiga antelope (*Saiga t. tatarica*) in Kazakhstan, showing the approximate range of the three populations and overall direction of migrations (north in summer, south in winter) (arrows) (Courtesy of Steffen Zuther, ACBK).

Saiga was once an important economic resource through sales of meat, hide and horns, the latter used in traditional Chinese medicine. During the Soviet period, the species was reasonably managed, for instance, the period 1980-1990 was a time of relative stability for saiga , with a global saiga population estimated at around 1 million, in spite of commercial hunting being a common practice (Bekenov et al*.,* 1998; Milner-Gulland et al., 2001). With the breakup of the Soviet Union in 1991-1992, a collapse in funding and infrastructure for saiga management, combined with a rapid economic disintegration in rural areas, led to uncontrolled large-scale poaching for meat and horns, resulting in a dramatic decline of the saiga population by more than 95% in less than one decade (Milner-Gulland et al., 2001), see Graph 1. Since then the species has received considerable international attention, having been included in 1995 in the Appendix II Species of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) to prevent the illegal trade of saiga horns. Additionally, Saiga antelope *(S. t. tatarica)* was classified as a critically endangered species in the IUCN red list of threatened species (Mallon, 2008) and listed in Appendix II of the Convention on Migratory Species (also known as the Bonn Convention) in 2002 (CMS, 2009). A Memorandum of Understanding (MoU) concerning the "Conservation, restoration and sustainable use of the Saiga antelope (*Saiga t. tatarica*)" was signed by Kazakhstan, Uzbekistan and Turkmenistan in 2006 (CMS, 2006). Russia and Mongolia joined in 2009 and 2010 respectively (CMS, 2010).

Graph 1 - Saiga population changes in Kazakhstan over the past three decades. 1980-2000 data from Milner-Gulland et al. (2001); 2001-2006 estimates from CMS (2006). 2003-2014 data from annual aerial surveys in Kazakhstan (Duisekeev & Sklyarenko, 2008; Grachev, 2012; Grachev, 2013; ACBK's unpublished data, 2014).



As a result of all conservation efforts and protection measures undertaken over the last decades, including livelihood interventions, anti-poaching actions, habitat protection in some areas, public awareness raising, population monitoring (CMS, 2006), combined with the rapid growth potential of the species under favorable conditions (Bekenov et al., 1998; Kühl, 2008), saiga populations have shown signs of recovery in some cases (Graph 1). Annual aerial surveys are conducted in Kazakhstan since the 1950s by the Institute of Zoology of the Kazakhstan Academy of Sciences, with the involvement of different conservation organizations (Bekenov et al., 1998), recently the Committee of Forestry and Wildlife of the Ministry of Agriculture of the Republic of Kazakhstan (CFW), Okhotzooprom State Enterprise and the Association for the Conservation of the Biodiversity of Kazakhstan (ACBK). The aerial surveys are carried out in spring (April), when saiga form large migratory herds, using an aerial transect counting method covering their entire range. According to the available data, the overall population in Kazakhstan has increased in recent years, with a last estimate at 256,000 individuals occupying the range in 2014, compared to 137,500 in 2012 and only 21,100 in 2003 (Grachev, 2012; Grachev, 2013), with the Betpak-Dala population the largest of all by 2014 - representing over 85% of the individuals in the country. This population was the first to be particularly affected by poaching in the late 1990s due to its location nearest human populated areas, having been reduced by 2000 to about 4% of the mean size in the period of 1980-1990, estimated at 375,600 (Milner-Gulland et al., 2001). The Ural population has shown an upward trend in the last few years, after having suffered a marked decrease in 2010-2011 (Graph 1) due to a disease outbreak ascribed to *Pasteurella multocida* (Grachev & Bekenov, 2010; Duisekeev, 2011). In the Ustiurt population, saiga numbers have declined from year to year. Estimates for this population were of 5,400 animals in 2013 and less than 2000 in 2014 (Grachev, 2013; ACBK's unpublished data, 2014) that is very low compared to 17,800 in 2006 (CMS, 2006). This may be a result of ongoing poaching pressure and disturbance in its range. Therefore the data presented above suggests that although there has been some progress in the restoration of the saiga population size in Kazakhstan over the last 10 years, saiga are still present in small numbers compared to the past, making them particularly vulnerable to stochastic events, such as infectious disease epidemics or extreme weather changes, so extinction is still a very real possibility.

1.3 PAST AND PRESENT EVENTS OF NATURAL MASS MORTALITY IN SAIGA

Saiga population sizes have been strongly affected by climate variability and diseases (Bekenov et al., 1998). Major fluctuations attributed to extreme weather conditions (*dzhuts* and droughts) were reported in the past, especially in the 1940-1970s. For instance, in the winters 1971–1972 and 1974-1975, respectively 400,000 and 100,000 saiga died in the southern part of Betpak-Dala (Fadeev & Sludskiy, 1982). Males weakened by the rut and juveniles were particularly affected by these harsh winters (Bekenov et al., 1998). According to published data, there is no evidence of mass mortality caused by extreme climate since 1993-1994 in Kazakhstan.

Significant declines in saiga population have also occurred as a result of large scale disease outbreaks associated with massive mortality. Six outbreaks of Foot and Mouth Disease (FMD) were recorded between 1955 and 1974 in Kazakhstan. The most serious occurred in 1967, resulting in the deaths of about 50,000 calves (Bekenov et al., 1998). In response to the FMD outbreaks, mass vaccination of livestock for FMD within the saiga range area was carried out in the 1970s and since then no outbreaks were reported in saiga . After the collapse of the Soviet Union, vaccination became more sporadic and outbreaks in livestock were again reported (Lundervold, 2001). Fortunately, the frequency of contacts between domestic livestock species and saiga became lower since Kazakhstan's independence, presumably as a combined effect of a major decrease in human density and livestock populations size in rural areas (Robinson & Milner-Gulland, 2003), which may have mitigated the odds of FMD transmission. A past serological survey carried out in 1997-1998 showed seropositive to FMD in livestock, mostly due to vaccination but some due to infection; however, saiga in the Betpak-Dala and Ustiurt population tested negative for antibodies to FMD (Lundervold, 2001), suggesting that the population was susceptible to infection. Yet recent surveys revealed positive results for antibody detection in the Betpak-Dala population, which contradicts earlier findings (M. Orynbayev, unpublished data, 2014). According to the Animal Health World Organization (OIE) the following regions of Kazakhstan: Akmola, Aktobe, Atyrau, west

Kazakhstan, Karaganda, Kostanay, Mangystau, Pavlodar and north Kazakhstan, are considered FMD free zones where vaccination is not allowed (OIE, 2014). FMD outbreaks were reported in cattle in May and June 2013, in east Kazakhstan, Tarbagatay region, near the border with China, a territory which is distant from the saiga range (OIE, 2013).

A number of events of massive mortality in saiga were also reported in the 1980s: in May 1981, over 70,000 saiga (mostly adult females and calves) of the Betpak-Dala population died; in February-March 1984, in the Ural population, more than 100,000; in May 1988 another outbreak during the calving season caused over 250,000 deaths in the Betpak-Dala population (Fadeev & Sludskiy, 1982; Bekenov et al., 1998). All outbreaks were officially attributed to *Pasteurella sp*., though the causation has been disputed. For instance in 1988, a hypothesis of intoxication when grazing close to a military base was raised by some senior scientists at the Kazakh Veterinary Science Research Institute (Lundervold, 2001).

Since the massive poaching-related saiga population decline in the 1990s, relevant mortality events were reported in the Ural population, causing the loss of about 75% of the population in May 2010 (~12,000 animals) and of an affected subpopulation in May 2011 (~500 animals), at about the same location. The initial diagnosis of Pasteurellosis as the primary cause of these two outbreaks was based upon the isolation of *Pasteurella multocida* from tissue samples taken at post-mortem, but unsupported by pathological or epidemiological evidence. By this fact, the mono-factorial causation attributed to both outbreaks has been called into question. Retrospective analysis suggest the hypothesis of a ruminal dysfunction associated with the ingestion of unusually rich pastures during post-calving in certain areas, causing a syndrome of bloating and respiratory failure, resembling atypical interstitial pneumonia, “Fog fever” (Kock, 2001; Kock et al*.,* 2011; Sapanov, 2011; Dancer et al., 2012). However, the isolation of *Pasteurella multocida* is not in dispute and the bacteria might have been the final piece of a multifactorial complex puzzle given that this microorganism is opportunist (Kock, 2001; Kock et al*.,* 2011). Following these events in Western Kazakhstan, further episodes of increased mortality were notified in May 2012 and in September 2013, this time involving the Betpak-Dala population, with preliminary laboratory results pointing to Pasteurellosis, according to the official statements of CFW (formerly known as Committee of Forestry and Hunting or CFH; Zuther, 2012; Saiga Conservation Alliance, 2013). The CFW refers that about 1000 saiga were affected on each event. On the May 2012 outbreak, a number of animals with locomotor disturbance (paresis) was reported. Initially a mineral-related condition, such as hypocalcaemia, was suspected. Later, retrospective assessments suggest that those cases were probably due to calving problems as no mineral abnormalities were confirmed (M. Orynbayev, unpublished data, 2014).

After the completion of the fieldwork, as part of this study, a remarkable die-off event occurred during the 2015 calving season, devastating the majority (> 200,000 animals) of the Betpak-

Dala population (CMS, 2015). Comprehensive investigations, involving local and international experts, were conducted to establish a definitive aetiology and eventual co-factors for the massive mortality. Experts tend to agree on a multifactorial aetiology, primarily haemorrhagic septicaemia caused by *Pasteurella multocida* (type B), complicated by secondary factors, which may include clostridial enterotoxaemia. Scientists also consider the involvement of an environmental trigger, as yet unknown, as these bacteria are known commensal, opportunistic pathogens (Kock, Zuther, Khomenko & Orynbayev, unpublished data, 2015). Haemorrhagic septicaemia is so far the official cause of death reported by the National Reference Centre for Veterinary to the OIE.

Figure 6 - Mass saiga deaths in Torgai region of Kazakhstan in 2015 (Courtesy of Steffen Zuther, ACBK).

1.4 The problem

The mass mortality events in Saiga antelope during the last decade have received enormous national and international concern, as thousands of animals of this critically endangered species have died. However, these incidents, somewhat unexpected, generally revealed a lack of coordination and technical capacity of the local institutions, resulting in less-than-efficient investigations and in the inability to obtain comprehensive conclusive diagnoses, particularly in the case of the outbreaks between 2010 and 2013 (Kock et al*.,* 2011; ACBK's unpublished report, 2013). As a response to the still-ongoing problems, a number of actions aiming to improve future interventions have been carried out in Kazakhstan. A governmental program for research on saiga health status and diseases was implemented by the Research Institute for Biological Safety Problems (RIBSP) in 2012, involving annual field research and monitoring of saiga herds; cooperation with international wildlife specialists was initiated, including the Royal Veterinary College (RVC), London, and veterinary training courses were promoted in

2013 by the UN´s Food and Agriculture Organization (FAO) and ACBK in cooperation with the Committee of Veterinary Control and Surveillance of the Ministry of Agriculture of Republic of Kazakhstan. With the help of the FAO and RVC, Standard Operating Procedures (SOP) for disease monitoring and outbreak investigation were established and are undergoing implementation in Kazakhstan (ACBK's unpublished report, 2013; Zuther & Shmalenko, 2014). This includes the development of a Rapid Response Unit (RRU), a multidisciplinary team prepared to promptly react in future cases of disease outbreaks or any other mass die-off events in Saiga antelope.

The field experience of the previous events, particularly during the calving season in 2012, revealed difficulties in identifying if the observed frequency of mortality was due to a disease outbreak or on the contrary it was within limits of the mortality expected at calving. This is partly a result of a lack of knowledge on the regular mortality pattern during the calving season. The establishment of acceptable levels of mortality (background mortality), as well as an understanding of its dynamics over the calving season for saiga , would enable rangers and scientists to suspect of an outbreak more promptly and to report it immediately, sparking investigations earlier and making them more efficient in disclosing the causes of increased mortality. Consequently the SOP recommend a combination of active and passive epidemiological surveillance of the saiga populations. This includes annual monitoring of calving aggregations for data collection on all carcasses, including natural deaths, given that the majority of main die-off events are seasonally associated with calving.

The knowledge on mortality of Saiga antelope (*Saiga t. tatarica*) during the calving season is very limited and mostly comes from investigations carried out in the Soviet times, such as the data obtained in the Kalmykia population in Russia in the late 1950s (Zhirnov et al., 1998) and in Kazakhstan in 1960-70s (Fadeev & Sludskiy, 1982). Information on the study population and the methodologies are scarcely provided, not to mention that most available literature is written in Russian. Bekenov et al. (1998), in their English-written theoretical review, report few data on calf mortality and even that only for the period 1989-1993, when the impact of anthropogenic factors was quite high. Another available reference on saiga is the study of Bayarbaatar (2011) focussed on juvenile mortality of *Saiga t. mongolica*, a very small Mongolian population that does not undertake large seasonal migrations and subsists under different habitat conditions. Furthermore, Bayarbaatar's study precluded evaluation of death at or around the time of birth, attributing most deaths to predation in that population.

There is therefore a need to monitor the calving aggregations in order to quantify and characterize the patterns of regular mortality of Saiga antelope in Kazakhstan, which will be of crucial help for the early detection of abnormal mortality, outbreak investigations, disease surveillance and management and conservation of the species in the future.

2 AIMS OF THE STUDY

This study aims to improve the understanding and contribute for the establishment of baseline information on the patterns of background mortality of the Saiga antelope during the calving period. In order to achieve this primary goal, the following specific objectives were set:

1) monitor a saiga calving aggregation and dynamics of births;
2) measure newborn saiga densities in the calving area and estimate mortality rate;
3) measure adult saiga carcass densities in the calving area;
4) investigate proximate causes of death in both newborn and adult saiga .

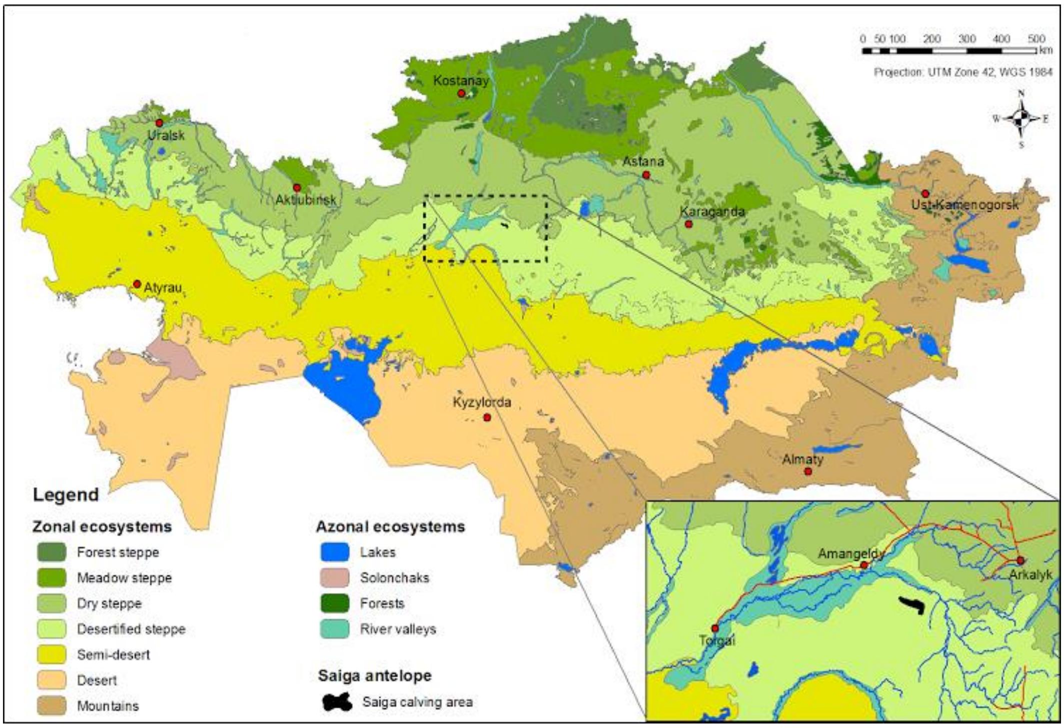
3 MATERIALS AND METHODS

The field research was carried out during the calving season of the Betpak-Dala saiga population in Kazakhstan, as part of a scientific expedition organized by the Research Institute for Biological Safety Problems (RIBSP), in collaboration with the Association for the Conservation of the Biodiversity of Kazakhstan (ACBK) and the Royal Veterinary College (RVC).

3.1 STUDY AREA

The study area is part of the Torgai plains, Kostanay region, Kazakhstan, where the main calving aggregation of the Betpak-Dala saiga population has occurred in recent years, according to data obtained from satellite transmitters (ACBK's unpublished data, 2014). It is part of the desertified steppe of Kazakhstan, mapped in Figure 7. The vegetation of the region is characterized mainly by Artemisia-short feathergrass steppe, sometimes combined with fescue or replaced by areas of orache communities. The climate is continental, with typically hot and dry summers, reaching 43ºC in July, and extremely cold winters when temperatures can drop as low as -44ºC in January. Thermal amplitudes may be high, both daily and seasonally, and strong gusts of wind can occur throughout most of the year. Climate is arid, with annual precipitation ranging approximately between 200 and 250 mm. Snow generally falls in the beginning of December, covering the plains until the end of March. Extreme weather events like *dzhuts*, characterised by an ice cover which develops over the snow and prevents animals from feeding, have not been observed for over 20 years, according to reports of locals of the Akkol village. Such extreme weather events were associated with increased mortality in saiga in the past, as previously mentioned (Bekenov et al., 1998).

Figure 7 - Main calving area of the of the Betpak-Dala saiga population in 2014, located in the northern desertified steppe of Kazakhstan (Courtesy of Steffen Zuther, ACBK).

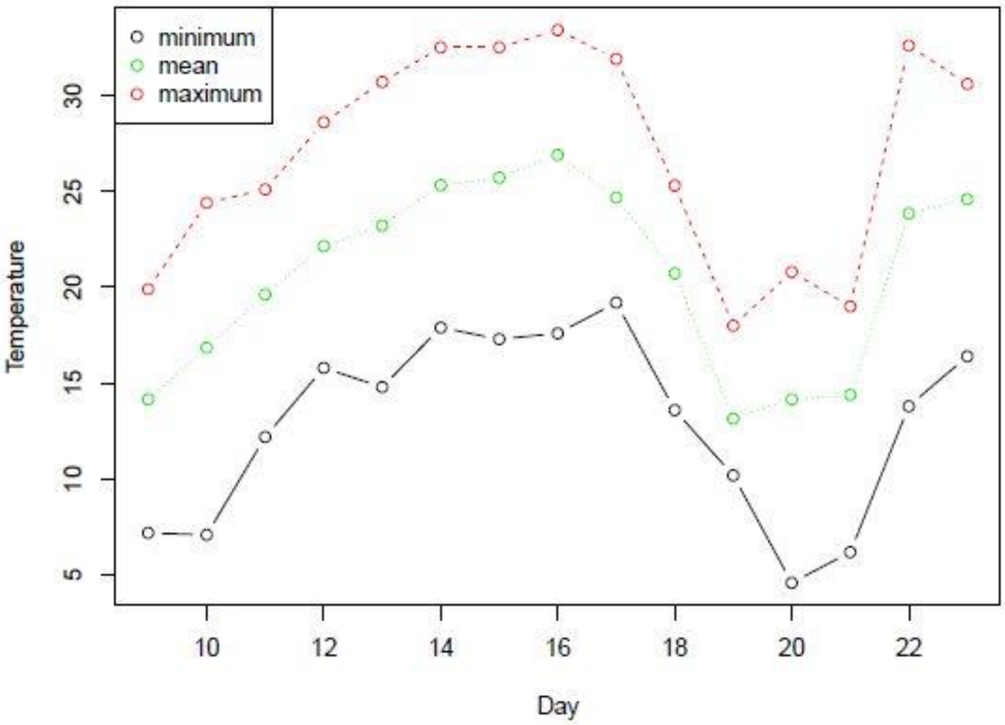


The smaller villages Kulik and Kyzylzhuldyz, located about 17 km and 25 km northeast, across the river Turgai, respectively, also constitute human populated areas nearby. The research team was based approximately 10 km to the west of the abandoned village, Kainar, only a few km away of the calving area. Domestic livestock occupying nearby range consists mainly of sheep, cattle and horses. Sheep and cattle are usually kept around the villages, while horses range freely, occasionally over long distances. At the time of the fieldwork a group of around 30 horses was seen in the area of the camp. Watering places include the mentioned perennial river Turgai, about 9-10 km north of the calving area, small streams and lakes, including the salty lake Murat and the lake Uirek. Additionally, several artificial ponds exist in the area, dug during the Soviet time to provide additional water supply for livestock. At the beginning of the fieldwork, a large group of saigas, mostly pregnant females, was observed near an artificial pond about 2 km north from the research camp.

The survey area was covered mostly by sagebrush-grass steppes. Dominant grass species include *Stipa sareptana*, *Festuca valesiaca, Koeleria cristata* and *Stipa lessingiana*. *Stipa capillata* can ocasionally be found, as well as *Agropyron fragile*. The composition of sagebrush communities includes *Artemisia semiarida* and *Artemisia lerchiana*. The calving area also included desert-steppe communities dominated by dwarf shrubs. The most distributed sagebrush community consists of *Artemisia gracilescens* and *Artemisia pauciflora*. Meadow vegetation generally develops in areas of higher relative humidity (ACBK's unpublished data, 2014). According to botanical investigations at calving time, saiga tend to feed mostly on herbs and some nutrient-rich grasses, passing over dwarf shrubs and feathergrass (ACBK's unpublished data, 2014). The energy and nutrient content of these plants may be the main reason for such selective feeding behaviour. This is supposed to be relevant when more energy and protein is required to launch and sustain lactation.

According to the meteorological station of Torgai, at the time of the fieldwork in May 2014, the average temperature reported was 20.6°C. The highest and lowest temperatures were respectively 33.4°C on day 16 and 4.6°C on day 20. Temperature dynamics throughout the study period is plotted in Graph 2 below. The strongest winds occurred on the May 9th and 13th (up to 18 km/h) and from May 18 to 21 (up to 32 km/h). According to field observations, wind speeds seemed significantly higher than the regional record at times, although this could not be measured. No precipitation was recorded by the station, but rainfall was observed in the field in the nights 19 and 20.

Graph 2 - Maximum, minimum and average daily temperatures (°C) for Torgai region of Kazakhstan, May 2014 (climate data obtained and adapted from Метеоцентр [Meteocenter], 2014**).**

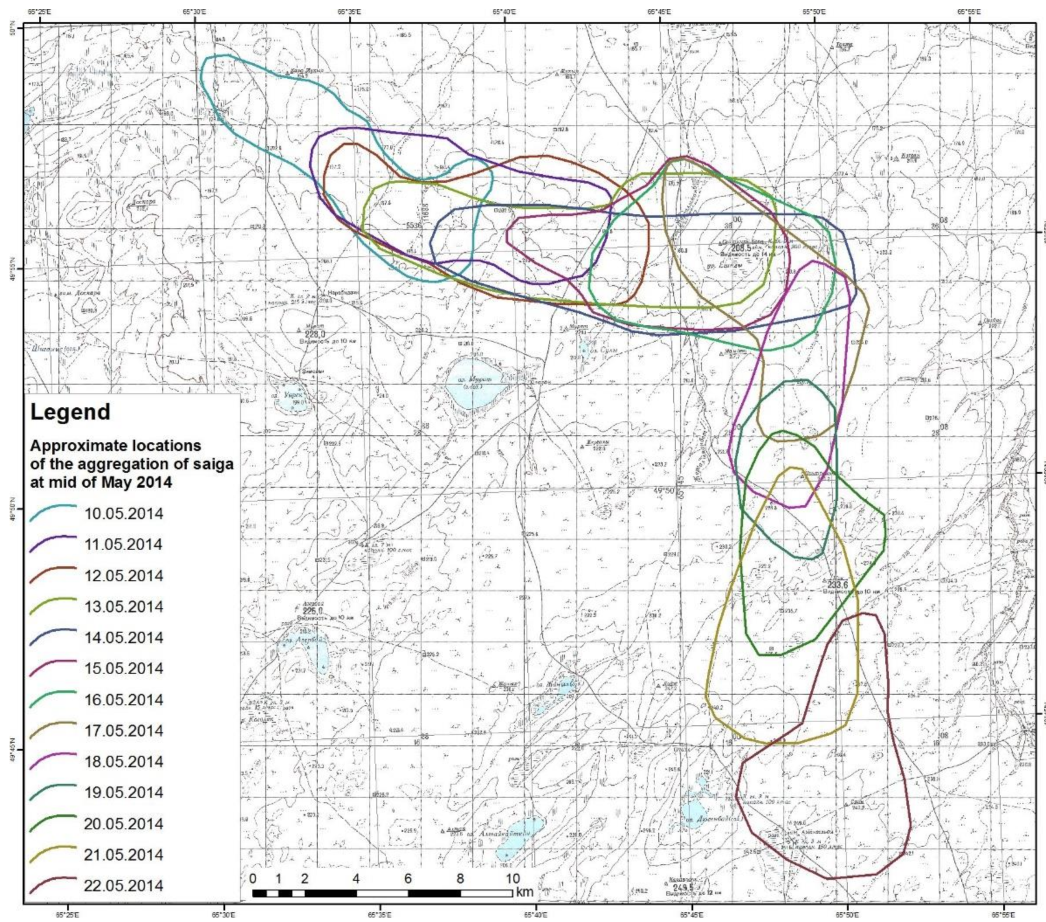


Wild fauna of the study area includes, among other species, wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), corsac foxes (*Vulpes corsac*), wild boar (*Sus scrofa*), souslik (*Spermophilus fulvus*) and raptors, such as the steppe eagle (*Aquila rapax*), golden eagle (*Aquila chrysaetos*) and cinereous vulture (*Aegypius monachus*). The only species sighted during the field research were steppe eagles, sousliks and red fox (one individual).

3.2 FIELD DATA AND SAMPLE COLLECTION

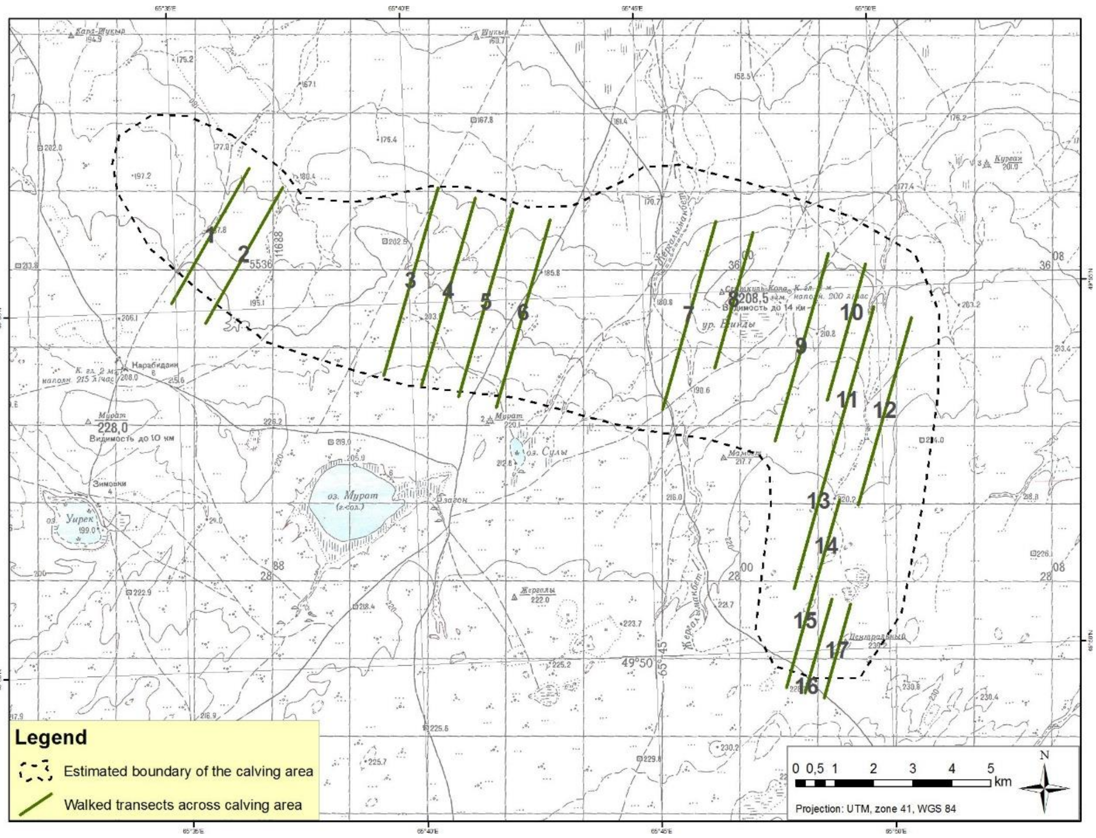
The fieldwork took place between May 9 and 22, 2014. The research team was based approximately 1.5-2 km south of the saiga aggregation. The main saiga aggregation site was determined based on the location data of five mature females previously tagged with satellite transmitters for population monitoring purposes by scientific experts of ACBK. On May 10, a first observation of the saiga concentration was made with a vehicle (60 km drive). The borders and the area of maximum concentration of animals were established. Daily observation was performed by vehicle to identify the location of the herd and, in particular, the direction of its movement. This information along with the satellite data of the tagged animals was used to plan straight line transect routes across the calving site, which varied daily in position according to the movement of the herd. The approximate locations of the main herd over the research period are shown on the map of Figure 8, where it can be observed that the herd came from west and moved eastwards, turning south on May 17, when it was located east of the abandoned village Kainar.

Figure 8 - Approximate locations of the main calving aggregation of the Betpak-Dala saiga population, from May 10th to 22nd, 2014. The locations were determined based on direct observations in the field as well as on telemetry data of tagged animals (Courtesy of Steffen Zuther, ACBK).



After the first signs that the birth period had commenced, GPS-guided transects were walked through the calving area, with systematic observations and recording of any live or dead saiga. A total of 17 transects were walked across the calving grounds (Figure 9), from May 12th to 21st, except on day 19 when transects were not conducted, due to time and resource constraints, but some previous areas were revisited. Each day, two transects were walked by three observers, 10 metres apart from each other, inspecting 5 metres to the left and to the right, covering a total 30-metre width.

Figure 9 - Transect routes across the estimated calving area of the Betpak-Dala saiga population, carried out by the author's team. The figure shows that transects (numbered from 1 to 10) were walked from the west to the east of the calving area, perpendicular to the movement of saiga aggregation, from May 12th to 16th. From May 17th onwards, when the herd started to move south, transects (numbered from 11 to 17) were walked in the direction of saiga movement (Courtesy of Steffen Zuther, ACBK).



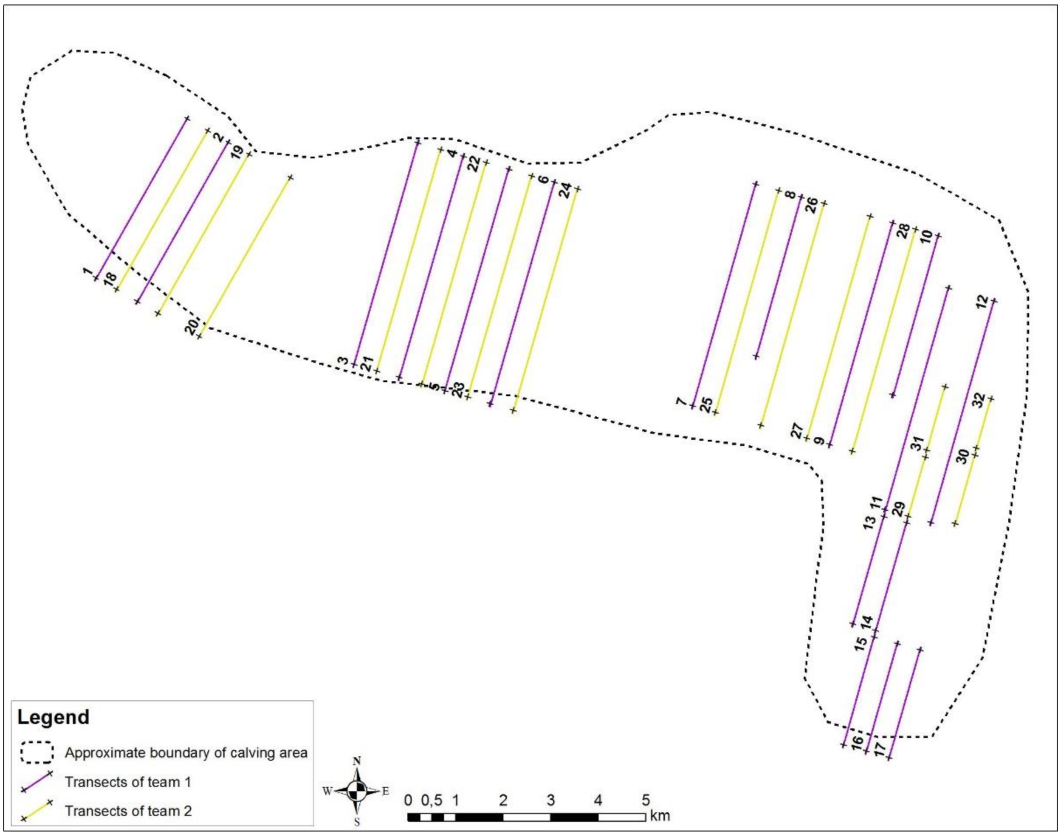
The length of each transect was meant to be 4-5 km, depending on the width of the aggregation, but this distance was not always achieved, mainly due to time constraints. Mean length of transects was 4.6 km (SD 0.59) from May 12th to 17th and 2.5 km (SD 0.00) in the last three days (Table 1). Generally, a distance of 1000 metres between each two transects was established, except on May 21st, when only one transect was walked by the team.

Table 1 - Transect sampling routine by the author's team at the main calving area of the Betpak-Dala population, from May 12th to 21st, 2014.

Time period	Number of transects	Total length (km)	Average length (km)	SD (km)
12th-17th May	12	55.26	4.60	0.59
18th, 20th-21st May	5	12.50	2.50	0.00

In addition to the 17 transects walked by the author´s team (named "Team 1"), a further 15 transects were carried out in parallel, approximately 500 metres away, by another group in the field (designated ahead in this study as "Team 2"). Figure 10 below illustrates the transect routes of both teams in the investigated calving area.

Figure 10 - Schematic view of the investigated calving area and the transect routes of both independent teams of observers. Each transect is numbered at the starting point.



The mean length of transects of Team 2 was 4.73 km (SD 0.59) from May 12th to 14th and 1.46 km (SD 0.18) from May 17th to 18th (Table 2). No transects were walked from the 19th to the 21st by this group.

Table 2 - Transect sampling routine of Team 2 at the main calving area of the Betpak-Dala population, from May 12th to 18th, 2014.

Time period	Number of transects	Total length (km)	Average length (km)	SD (km)
12th-16th May	11	52	4.73	0.47
17th-18th May	4	5.85	1.46	0.18

3.3 SAIGA CALF CAPTURE AND HANDLING

All the saiga calves in an aggregation are born within a relatively short time period (5-8 days) and as they generally can only be caught up to two days-old (Bekenov et al., 1998; Lundervold, 2001), timing is critical when sampling calves. During this initial period after birth, calves remain lying down at the birth places and no special immobilization method is needed to approach them. Thus, saiga calves, mostly up to 2 days-old, observed along the transects were caught by hand and carefully handled for sexing, weighing, assessment of hydration (skin fold) and observation of any physical injuries, evidence of disease or abnormalities. Older calves (> 2 days old) generally could not be captured, as they were more alert and able to run away quickly at the team's approach.

All saiga calves found along the walking transects were recorded in a GPS device as a point with coordinates, including those which escaped. In this case, their approximate location was recorded as well as possible through the observation of fresh tracks on the ground.

Body weight was determined by hanging the calf on a digital scale, and noted to the nearest 20 g. Monitoring of saiga calves was performed as quickly as possible, with the involvement of local scientific experts. During all procedures, the eyes of the calves were covered and silence was assured to minimize disturbance. Mortality attributed to post capture abandonment has not been reported in previous studies involving similar procedures in saiga calves, nor in this study, based on field observations and camera trap records.

3.4 SAIGA CARCASS EXAMINATION

Saiga carcasses were recorded (as a point in a GPS device) and examined post-mortem on site in order to determine proximate causes of death. Necropsy was guided by the project supervisor, generally consisting of a systematic external and internal examination of the body and organs (except nervous system) following the methods described in the manual of necropsy of wild animals by Munson (1970). Heavily decomposed or scavenged carcasses were not necropsied. General observation included carcass position, general area of soil and vegetation around the carcass, signs of struggling, predation traits and placentae. All post-mortem findings were recorded in a necropsy report form for further analysis (Annex 1). Sex and post-mortem body weight of saiga calves were also registered. In the case of adults, an assessment of the age was made, based on tooth development and general appearance of the animal (e.g. body size, horns, etc.). The primary criteria for saiga age estimation was described by Bannikov (1963).

3.5 LABORATORY PROCESSING AND HISTOPATHOLOGY

Samples of organs and tissues, generally including lung, thymus, thyroid gland, liver, spleen, kidney, adrenal gland, abomasum, intestine, heart and skeletal muscle were collected from recently deceased animals for histopathological examination. Tissues were fixed in 10% buffered formalin, submitted to a laboratory at the Royal Veterinary College in London for routine processing and evaluated for histological abnormalities by the author with the support of Pathology professors.

3.6 DATA PROVIDED BY OTHER RESEARCH TEAM IN THE FIELD

Additional newborn calf data, including body weight, sex and location (GPS coordinates), and saiga carcass data (sex and GPS coordinates), collected by Team 2 working in the area for other research purposes, were kindly made available for analysis in this study. Necropsies were not performed on these animals, but data from general observations and photographic records were analysed and, whenever possible, a cause of death assigned. Post-mortem body weights were also not assessed in these cases.

3.7 QUANTITATIVE DATA ANALYSIS AND STATISTICS

After the fieldwork, the data was transferred to electronic format and prepared for analysis. ArcGIS was used to map data points (GPS coordinates) and to assess the location of the animals as inside or outside the transect area. Only data points inside transects were used for quantitative assessments (as density calculations and calf mortality rate).

Microsoft Excel 2010 ® was used to process the data and to conduct simple descriptive analyses. Statistical tests and models with corresponding diagrams were performed using R. The t-test was applied to test significance between means and the Pearson´s chi-squared test (χ^2) was used to test the significance between proportions, in which $p<0.05$ was considered significant. A simple lowess smoother (LOWESS; Cleveland, 1979; Cleveland, 1981) and a Generalized Additive Model (GAM; Wood, 2006) were used to explore temporal trends of a variable (density) and any relationship between a variable (densities) and covariates (as temperature variable).

4 RESULTS

4.1 SEX RATIO OF SAIGA CALVES

A total of 856 saiga calves (823 alive and 33 dead), sampled within the areas covered by transects, were evaluated for sex. The analysis excludes three dead calves, of which sex records were missing. Overall, the proportion of male and female calves was 54.56% and 45.44% respectively. Significantly more males were captured ($p<0.01$). The results of both teams, separately, are fairly consistent, as shown in table 3.

Table 3 - Data on sex ratio of saiga calves (n=856) in the Betpak-Dala population, 2014.

Data	Sample size	Male		Female		Ratio
		N	%	N	%	
Team 1	381[(1)]	208	54.59	173	45.41	1:0.83
Team 2	475[(2)]	259	54.53	216	45.47	1:0.83
Total	856	467	54.56	389	45.44	1:0.83

[(1)] Recording of sex missing for one calf. [(2)] Recording of sex missing for two calves.

When analyzing dead animals separately, the proportion of female carcasses recorded on transects was higher than that of males (Table 4). The difference between sex ratio of dead calves and overall sex ratio was statistically significant ($p<0.05$).

Table 4 - Data on sex ratio of dead calves (n=33) in the Betpak-Dala population, 2014.

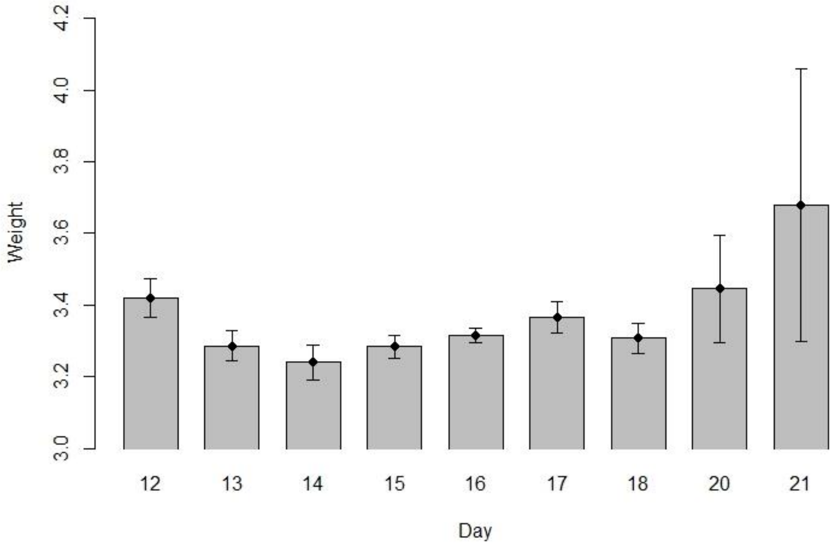
Data	Sample size	Male		Female		Ratio
		N	%	N	%	
Team 1	23[(1)]	7	30.43	16	69.67	1:2.29
Team 2	10[(2)]	4	40.00	6	60.00	1:1.5
Total	33	11	33.33	22	66.67	1:2

[(1)] Recording of sex missing for one calf. [(2)] Recording of sex missing for two calves.

4.2 BODY WEIGHT OF SAIGA CALVES

A total of 941 saiga calves (516 males and 425 females) were evaluated for body weight, including 118 calves located outside the areas covered by transects. Overall the mean body weight (±SE) of saiga calves was 3.31 ± 0.01 kg, varying from 1.96 to 4.46 kg. The mean body weight of calves monitored on each day of the survey period is shown in Graph 3. The values do not vary much, except for the beginning (May 12) and end (May 20-21) of the investigation, when the mean body weights were higher, although not significantly.

Graph 3 - Mean body weight of saiga calves (kg), with respective error bars (SE), in the Betpak-Dala population, 2014.



The mean body weight (±SE) of male and female calves was 3.42 ± 0.02 kg (range 2.26 - 4.46) and 3.18 ± 0.02 kg (range 1.96 - 4.20), respectively. The mean body weight of male calves was significantly higher than that of female calves (Table 5).

Table 5 - Comparison of the mean body weight ±SE in kg of male and female newborn saiga in the Betpak-Dala population in May 2014 (n=941), with respective 95% level confidence interval for each sex.

	N	Mean	Lower limit	Upper limit	*p*
Male	516	3.42 ± 0.02	3.39	3.46	< 0.001
Female	425	3.18 ± 0.02	3.15	3.22	

A total of thirty five calves were weighed post-mortem. Carcasses found heavily scavenged or decomposed are not included. Dead calf body weight was, on average, 2.71 ± 0.11 kg, varying from 1.20 to 4.08 kg. Overall, dead calves were significantly lighter than live calves, as shown in Table 6. Mean body weight for dead male calves (2.77 ± 0.14 kg, range 1.28 - 4.08) was not significantly higher than that of dead females (2.66 ± 0.16 kg, range 1.20 - 3.88).

Table 6 - Comparison of the mean body weight ±SE in kg of dead and live saiga calves in the Betpak-Dala population, 2014. A 95% confidence interval is presented for each group.

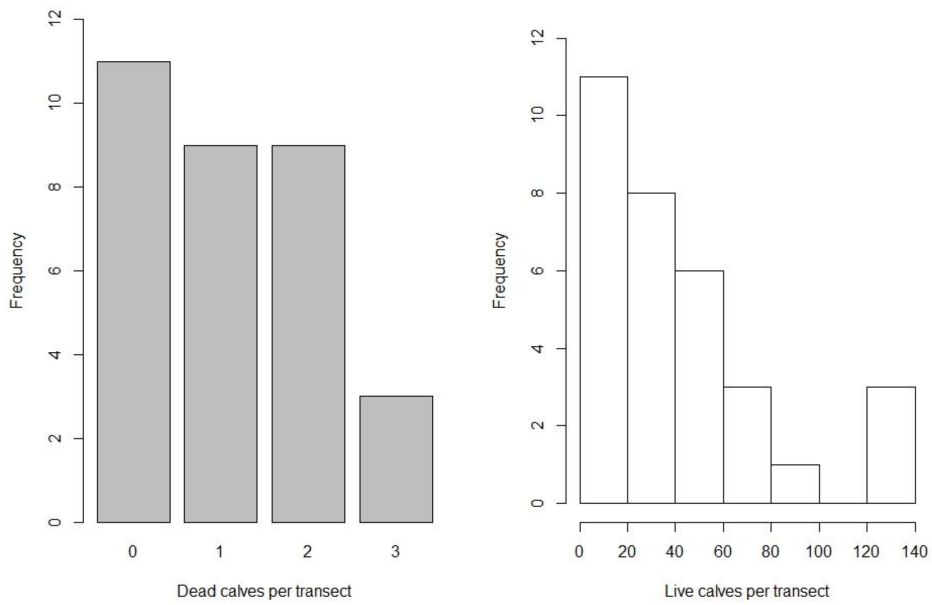
	N	Mean	Lower limit	Upper limit	*p*
Alive	941	3.31 ±0.01	3.29	3.34	< 0.001
Dead	35	2.71 ±0.11	2.50	2.93	

4.3 TRANSECT COUNTS OF SAIGA CALVES

A total of 1353 saiga calves (1317 alive and 36 dead) was recorded within the transects by the two independent teams, in the survey period May 12-21, 2014. The number of dead calves per transect was 1.1 on average, ranging from 0.0 to 3.0 calves, while the average number of live animals per transect was 41.0, varying from 2.0 to 140.0 calves. Note that these results on transect counts do not take into account the differences in the sizes of the transects.

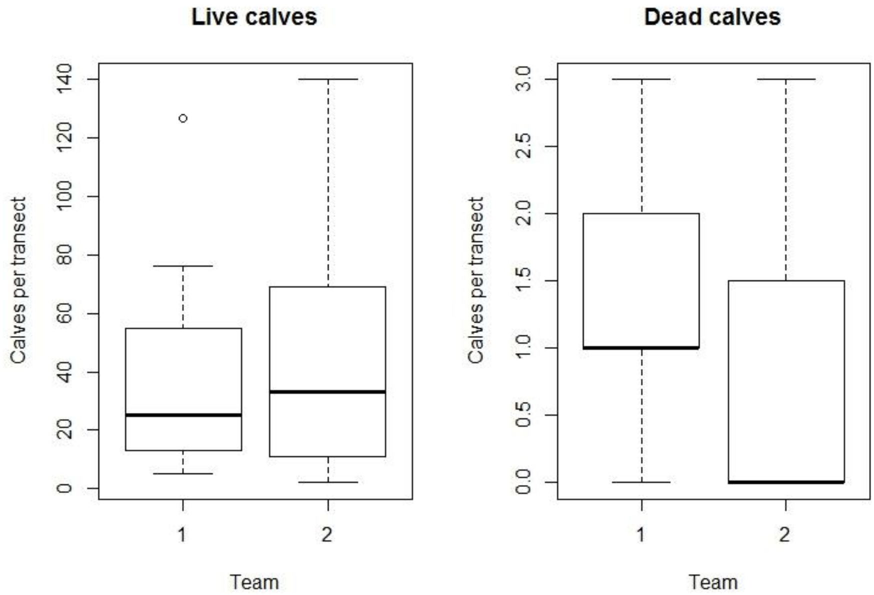
The numbers of dead and live calves per transect and respective frequencies are plotted below (Graph 4). The left barplot shows that 11 transects had no dead calves, with 9 transects with 1 and 2 dead calves each, and only 3 transects with 3 calves. The histogram plot on the right presents the information for live animals, with less than 100 animals typically found per transect and with 3 transects presenting more than 120 calves.

Graph 4 - Boxplots comparing the number of live and dead saiga calves per transect seen by the two teams in the field (Teams 1 and 2).



The counting results of both independent teams were pooled under the same object in order to facilitate the analysis. When explored graphically whether the two teams saw similar numbers of calves, no obvious differences arose (Graph 5), which is reasonable, as otherwise we would have to consider any bias associated with one of the teams.

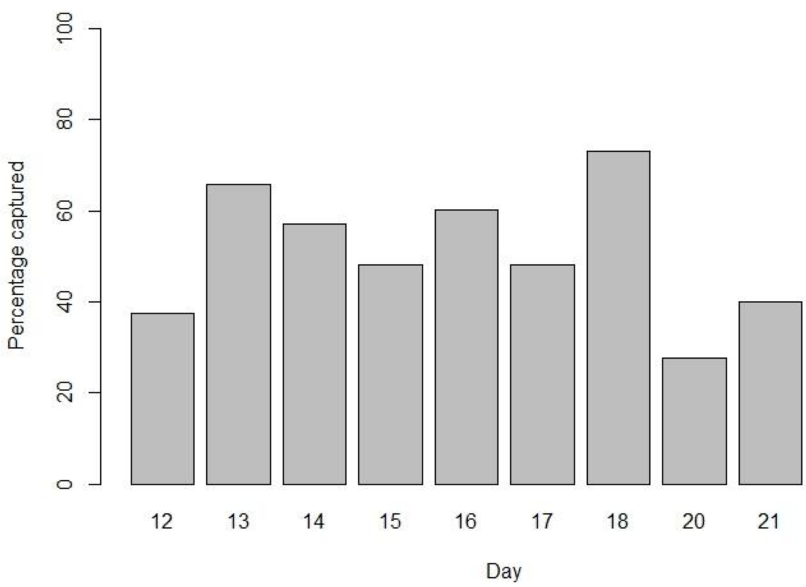
Graph 5 - Boxplots comparing the number of live and dead saiga calves per transect counted by the two independent teams in the field (Teams 1 and 2).



4.4 CAPTURE RATE OF SAIGA CALVES

Of the total of 1317 calves located on transects, 823 (62.5%) were captured; the remaining escaped. An overview of the daily capture rate is given in Graph 6. The proportion of captured calves (relative to the total recorded daily) was always above 50%, except for the beginning (May 12) and the end (May 20-21) of the investigation.

Graph 6 - Proportion of captured calves (%) relative to total number recorded on the transects per day.

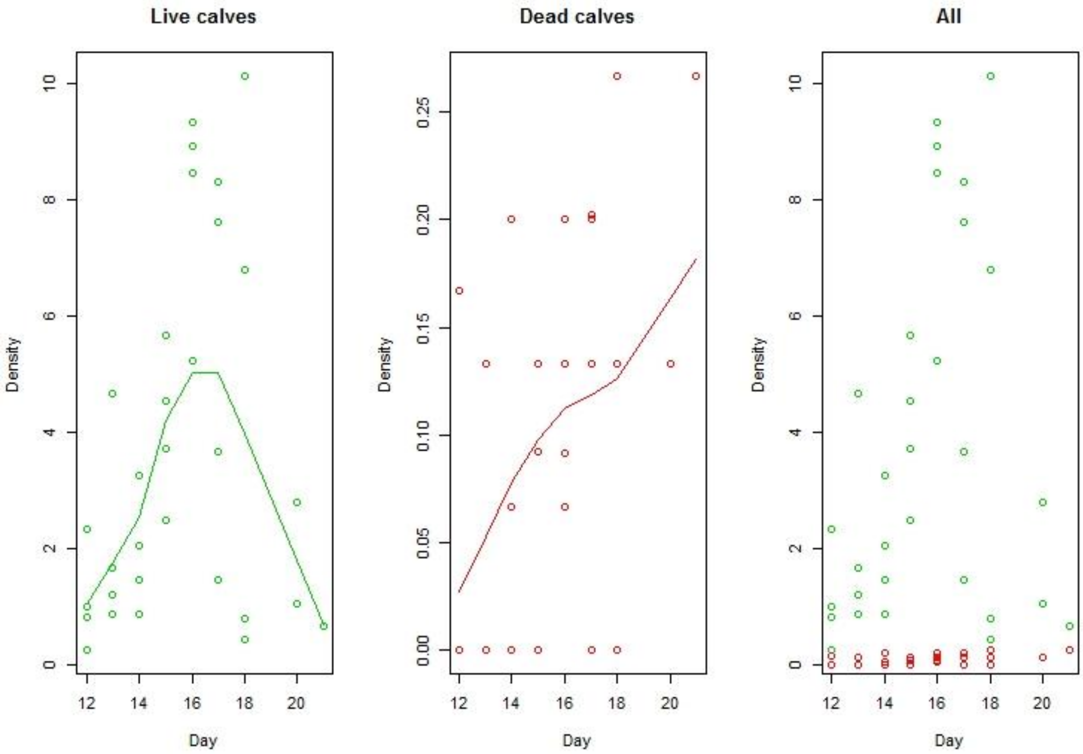


According to the overall capture results of the survey period (day 19 not included), of the total of 823 calves captured on the transects, approximately 23% were caught during the first three days (May 12-14), ~76% in about the middle of the survey period (May 15-18), and finally only 1% in the last two surveyed days (May 20 and 21).

4.5 DENSITY OF SAIGA CALVES

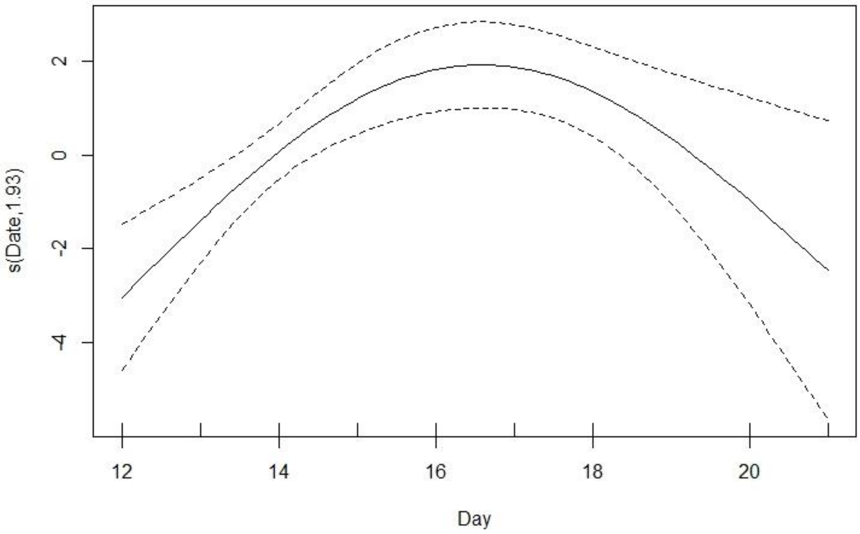
The density of live calves registered on the different transects ranged between 0.25 and 10.13 individuals per ha (25 to 1013 calves/km^2), while concerning dead calves, the range was 0.0 to 0.27 carcasses per ha (0 to 27 carcasses/km^2). In order to visualize temporal changes of the densities of dead and live calves during the survey period, a LOWESS was used. Apparently, the number of live animals exhibits a mode in the middle of the survey period, while the number of dead animals shows a continued increase with a brief period of apparent stabilization halfway through the survey (Graph 7), although the data is far too sparse, in particular for the last three days, to allow us to draw strong inferences.

Graph 7 - Timeplots with loess smooth curves (continued line) of the density of live (◦) and dead (◦) saiga calves for the duration of the survey period in May 2014.

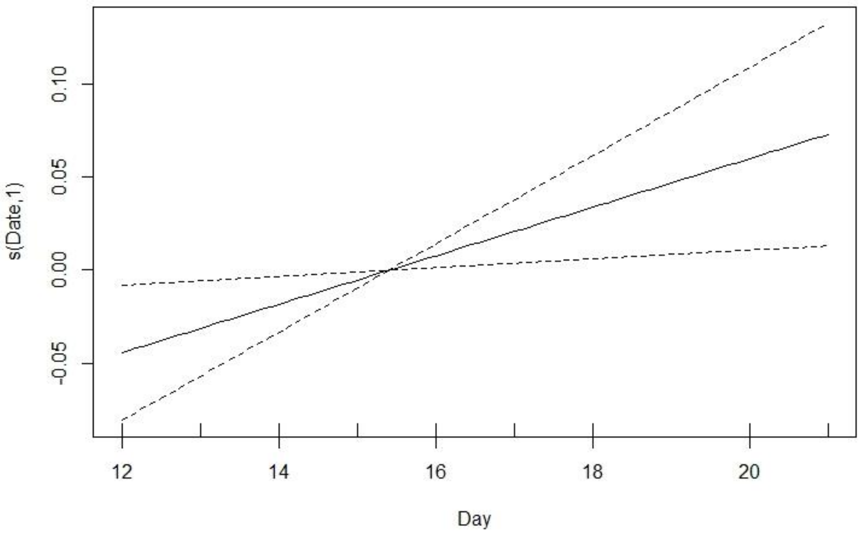


A GAM was further applied in order to provide a more refined analysis of densities as a function of available covariates, considering team and day, and treating live and dead calves separately. Statistically significant differences were not found across teams. The temporal trends in the densities of calves considered above with the LOWESS were considered significant with the GAM framework, at a level of $p<0.001$ for live calves and $p<0.05$ for dead. The shape of the smooth of day for live calves is plotted in Graph 8, whereas Graph 9 presents the smooth of day for dead calves, which in this case does not seem to require more than a linear term.

Graph 8 - Shape of the smooth of day for live calf density with GAM.



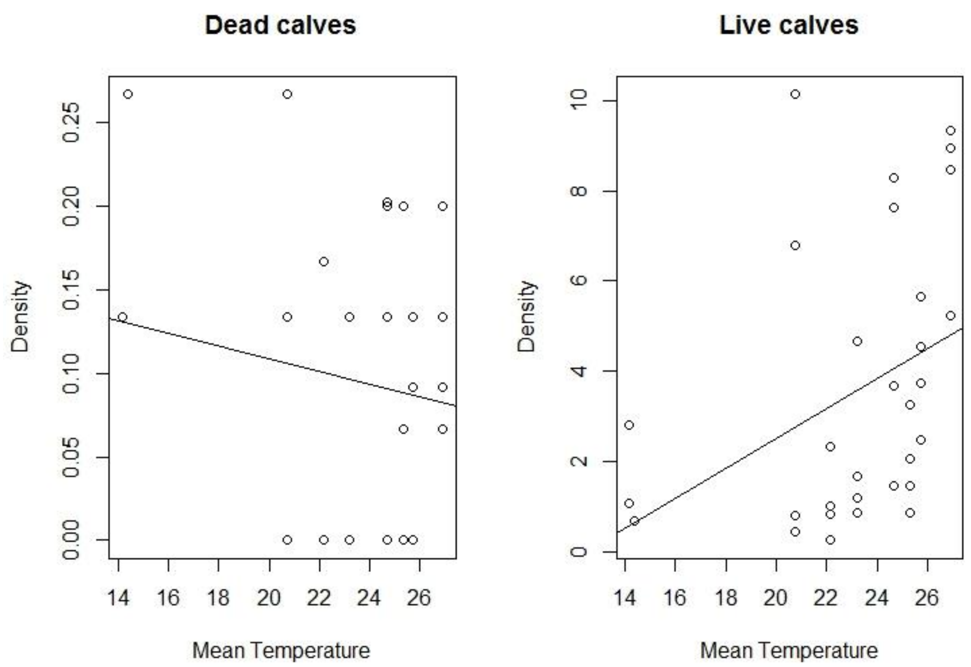
Graph 9 - Shape of the smooth of day for dead calf density with GAM.



4.6 CLIMATIC VARIABLE (TEMPERATURE)

Variations in weather conditions were detected over the study period, but appropriate measurements were not made in the field. According to the available climate data from the meteorological station of Torgai for the duration of the fieldwork period (May 9-22), air temperature was rising over the first days, reaching the highest levels around day 16 (maximum 33.4ºC), and dropped rapidly towards the end of the study period, presenting a marked minimum (4.6ºC) on day 20 (review Graph 2, methods section). In order to explore if and how births and/or calf mortality events might relate to the environmental temperature, the available information on daily average temperatures was used to relate to densities of calves during the survey period (data represents an approximation to local temperatures at the calving site, since the respective weather station is about 150 km away). This component was treated separately from the rest of the analysis to further stress its exploratory nature. Average daily temperatures ranged from 14.2 to 26.9ºC in the period of May 12-21, when transects were performed and densities measured. Day 19 was excluded from this analysis, since no density measures were obtained for this day. Graph 10 shows the densities of dead and live calves in relation to average daily temperatures.

Graph 10 - Densities of dead and live saiga calves (individuals per ha) recorded by the two teams over the study period and respective average daily temperatures (°C).



A slight trend for higher densities of dead animals on days with lower temperatures can be noticed, as it is visible by the downward sloping of the plot's trendline (Graph 10), although it was not statistically significant. The analysis for dead calves was repeated using the minimum temperatures (range 4.6-19.2), in order to more appropriately verify the potential effect of cold temperatures, which produced the same results. Regarding data pertaining to live calves, higher numbers of newborn calves were recorded in higher temperatures (Graph 10). This tendency was statistically significant ($p<0.01$), when further examined with the GAM framework.

4.7 ESTIMATING WIDER AREA DENSITY AND MORTALITY

In order to estimate the mean density of either dead or live calves in the whole calving area, a weighted mean of all observed densities was calculated, weighted by the corresponding transect areas, as the result of a simple arithmetic mean could be biased by the variable length of the different transects. Thus, the estimated average densities (and approximate 95% confidence interval) were 0.1 (95%, CI 0.07 - 0.12) dead calves per ha and 3.49 (95%, CI 2.5 - 4.49) live calves per ha. Although the transects have only covered a small part of the whole calving area, which in total was estimated at 13144 ha, an attempt was made to assess the total number of calves (alive and dead) in the area. In order to achieve this, the average densities were multiplied by the total area of interest, resulting in the following estimates (and respective 95% confidence intervals): 1314 (95%, CI 920 - 1577) dead calves and 45938 (95%, CI 32824 - 59052) live calves.

When comparing the densities of dead calves and the total densities of calves recorded for the different transects, the proportion of dead calves is estimated to be 4.4%, with a 95% confidence interval (2.3 - 6.6). This corresponds again to a weighted average, in this case of all proportions of dead calves observed in different transects.

4.8 PROXIMATE CAUSES OF DEATH OF SAIGA CALVES

A total of forty-seven dead calves were found during the period of May 12th to 22nd, at the calving areas; twenty-three of which were located outside the areas covered by transects. Based on the results of post-mortem examinations and on general observations, calves were classified per presumptive cause of death, as described below, and summarized in Table 7.

Stillbirth

Stillbirths of unknown cause occurred in six of all calves (12.8%), more often females (ratio 4:2). Animals exhibited dark-purple, non-aerated lungs, and were coated with thick maternal mucus or had fetal membranes attached and a wet, fresh looking umbilical cord. They had a white to yellowish membrane on the soles indicating they had not stood or walked, and showed no recognizable signs of birth trauma or malformations. One of the calves had the amnion over the nostrils and mouth. In three of the calves, post-mortem decomposition was advanced; the placenta had been expelled together with one of them. Most cases were registered approximately midway through the survey period (Graph 11).

Dystocia

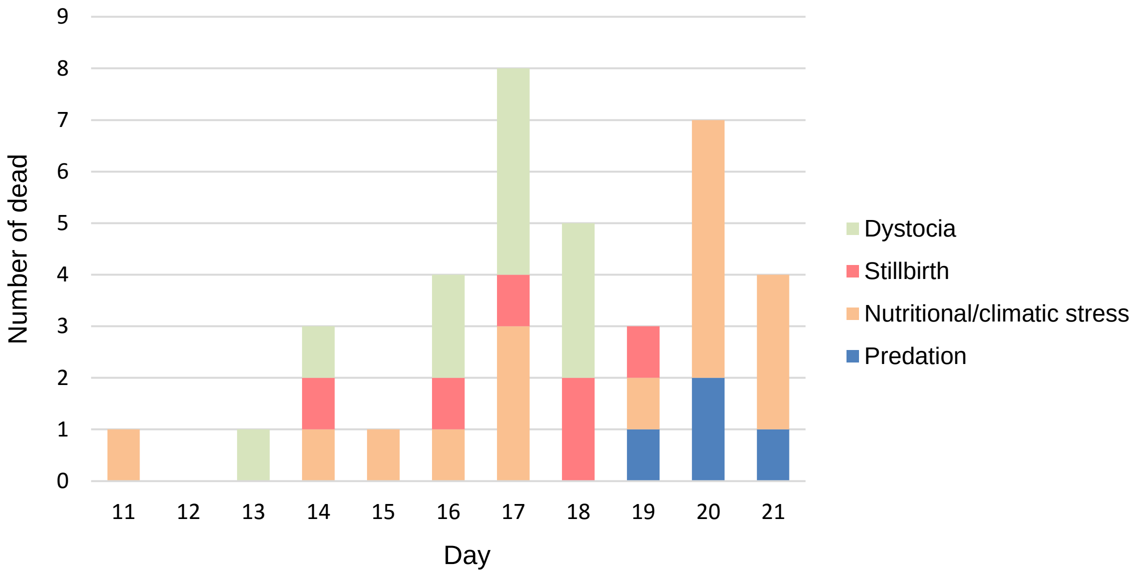
Six calves (12.8% of all losses) died subsequently to difficult or prolonged parturition, based on evidence of significant lesions consistent with birth trauma. The frequency was equal between male and female calves (ratio 3:3). Post-mortem examination revealed non-inflated to partially inflated lungs, lack of hoof wear, empty abomasum, body fat with no trace of having been metabolized and meconium not yet voided. Calves showed moderate to marked subcutaneous oedema over the head, sometimes extending down to the neck, or moderate subcutaneous oedema and bruising beneath the mandible and around the larynx (Figure 11b). Bruising of the free edges of the tongue was often noted (Figure 11c). Hepatic rupture and haemorrhage was present in one calf. Histopathological examination, carried out on one of the calves showing marked localized subcutaneous oedema, revealed complete pulmonary atelectasis (Figure 12b), multifocal hepatic haemorrhage (Figure 12c), consistent with trauma, and marked renal congestion. Some degree of organ autolysis (especially of the renal cortex) was noted.

Table 7 - Saiga calf losses and sex recorded by Team 1 in the Betpak-Dala population in May 2014 (n=47).

Categories	N	%	Male	Female	Unsexed
Stillbirth	6	12.8	2	4	
Dystocia	6	12.8	3	3	
Nutritional-climatic stress					
Malnutrition/ dehydration	7	14.9	2	5	
Starvation/ hypothermia	9	19.1	4	5	
Underweight/ underdeveloped	3	6.4	1	2	
Mother's death with calf inside:					
dystocia-related	5	10.6	3		2
predation-related	3	6.4	1	1	1
Predation	1	2.1			1
Scavenging	3	6.4		2	1
Inconclusive/unknown	4	8.5	2	2	
Total	47	100	18	24	5

In addition to the six cases mentioned above, further five calves (10.6% of losses) were found dead inside their mother’s birth canal and womb, which died from dystocia-related complications. One case of twin pregnancy and one case of singleton were confirmed. Two adult females were not inspected internally and therefore the total number of fetuses was not assessed. In all cases, a calf was found maldisposed in the birth canal, but not disproportionate in size relatively to their respective mothers. Thus, in total, difficult calving was responsible for 23.4% of all calf losses reported in this study. Similarly to stillbirths, most of these deaths occurred halfway through the survey period (Graph 11).

Graph 11 - Approximate distribution of deaths from dystocia, stillbirth, nutritional-climatic stress and predation over the survey period (n=37).



Nutritional/climatic stress

This group accounted for 34% of all calf losses, with deaths being attributed to nutritional deprivation and/or climatic stress, generally based on:

- evidence of body fat metabolism and absence of milk in the gastrointestinal tract;
- no other recognizable lesions that could justify death;
- field observations of potentially adverse weather conditions.

Calves are then further subdivided taking into account the prevailing weather conditions at the estimated time-window of their death.

a) Malnutrition/dehydration

Seven newborn calves (14.9% of all), mostly female (ratio 5:2), presumably died malnourished and dehydrated postpartum during days in which predominantly high temperatures were recorded (Graph 12). Most calves showed incomplete aeration of the lungs (Figure 13b), represented by few to many pink lobules contrasting with dark purple areas of atelectasis, and slight hoof wear. Post-mortem examination also revealed generalized dehydration, partial depletion of fat deposits, as indicated by coronary grooves completely devoid of fat or with reddened and gelatinous fatty remains, and lack of milk in the gastrointestinal tract (Figure

13b), with only small amounts of grass in a few cases. Meconium had not been fully expelled in most cases. In three of the animals, liver appeared congested and friable. No other significant macroscopic changes were observed. Histopathological examination was carried out on four calves within this category, having revealed partial pulmonary atelectasis (Figure 13c) and marked hepatic congestion in the three cases mentioned above, with one of them also showing moderate renal congestion; and extensive pulmonary atelectasis, with no other significant changes, in the fourth case.

b) Starvation/hypothermia

Nine calves (19.1% of all) presumably died due to starvation or hypothermia. These deaths apparently occurred during the last days of the survey period, when temperatures declined precipitously (Graph 12) and occasional periods of rainfall and strong winds were observed. Calves generally had fully aerated lungs (Figure 14b,c) and worn and sharp hooves. Post-mortem examination also showed an advanced depletion of body fat reserves, in most cases with all visible fat replaced by a shrunken, dark-purple soft or gelatinous tissue (Figures 14b, 15a,b), absence of milk in the abomasum (Figure 14b), sometimes with small amounts of grass in the still-developing forestomachs, and often dehydration. In three calves, occasional hepatic and renal congestion was noted. Histopathological examination, carried out on these three calves, revealed moderate to marked hepatic congestion (with discreet macro-vacuolar degeneration); mild to moderate renal congestion; and lack of intracellular lipid-vacuoles in the periadrenal brown adipose tissue, supporting severe brown fat depletion or atrophy (Figure 15c).

One of the calves was found in lateral recumbence curled up on itself and had failed to suckle, but, contrary to the previous cases, its fat metabolism was minimum, suggesting that death occurred soon after birth. No significant lesions or other indications that could explain the loss were identified. This calf was suspected to have died from cold exposure (lethal hypothermia), apparently too rapidly for there has been any depletion of its fat deposits.

Deaths from nutritional-climatic stress were recorded throughout all sampling period, but half of the cases were recorded in the last two days (Graph 11).

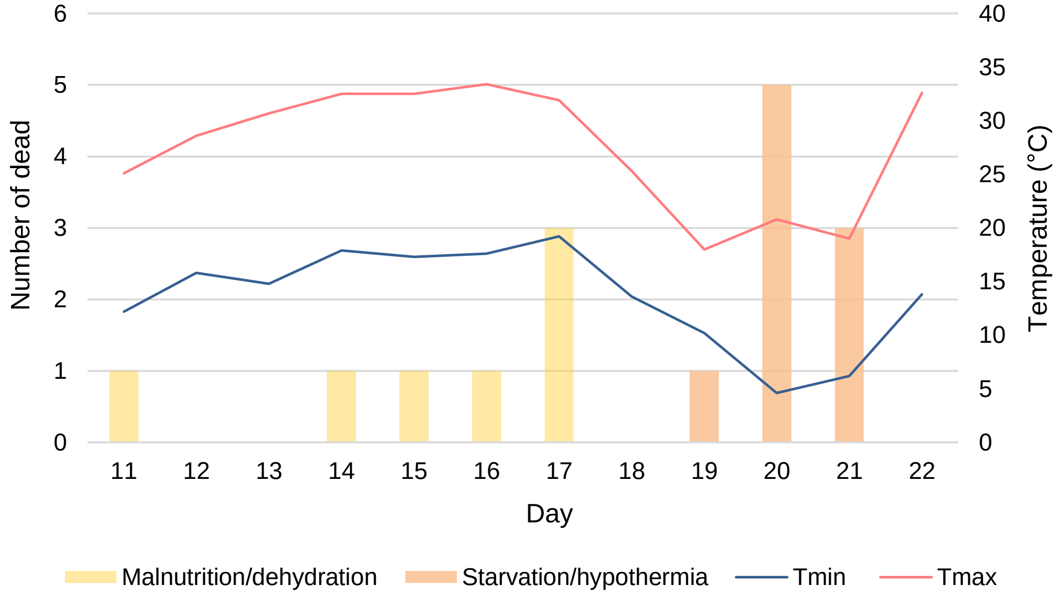
Underweight/underdeveloped

One male calf was extremely underweight (1.28 kg) and undersized (38.4 cm length from nose to rump), when compared to full-term newborn calves. It had partially aerated lungs, minimal hoof wear and had failed to suckle. Its hydration was normal and body fat metabolism minimal, however, suggesting it did not survive long after birth. Post-mortem examination also revealed a heart defect, patent ductus arteriosus. No other significant macroscopic changes were found. Histopathological analysis carried out on this calf showed partial pulmonary atelectasis, and no other significant findings. The extremely low body mass at birth, suggesting either prematurity or a twin/triplet birth, was deemed to have strongly limited this animal's survival. Two other female calves were also included in this group considering their very low body weight (on average 1.4 ± 0.20 kg), with no recognizable gross changes at post-mortem examination. This group accounted for 6.4% of all calf losses.

Predation

One dead calf (2.1% of all) showed clear evidence of a predator attack. Talon-inflicted puncture marks and haemorrhage were observed over the neck, showing it was attacked and dropped most probably by a raptor, such as an eagle. In addition, a further two calves were found dead inside females which had been attacked by a predator and another was suspected to have been directly pulled out from the vagina of the mother by the predator, most likely a wolf. These additional cases represented 6.4% of all deaths. In total, predation accounted for 8.5% of the losses. Predation-related deaths occurred at the end of the survey period, as illustrated in Graph 11.

Graph 12 - Deaths from nutritional/climatic stress (n=16) and minimum and maximum approximate temperatures for each day of the expedition (climate data obtained from Метеоцентр [Meteocenter], 2014).



Scavenging

Three carcasses (6.4%) were found heavily scavenged. A female calf, apparently scavenged by birds, showed the presence of maternal fluids on the coat and lack of hoof wear, suggesting stillbirth or death shortly after birth. The two remaining animals included in this group walked, as confirmed by their worn hooves, suggesting that they may have been attacked rather than scavenged, but both were completely devoid of internal organs, preventing firm diagnosis and classification.

Inconclusive/unknown

Four cases (8.5% of losses) were classified as inconclusive/unknown. In two cases, post-mortem examination did not show any recognizable substantial gross lesions that could explain death. Calves were found during warm days and both animals had fed, as indicated by the presence of milk in their gastrointestinal tract. Extensive histopathological examination was carried out on one of the calves, but without conclusive findings. The other two calves were found heavily decomposed and the cause of death could not be determined.

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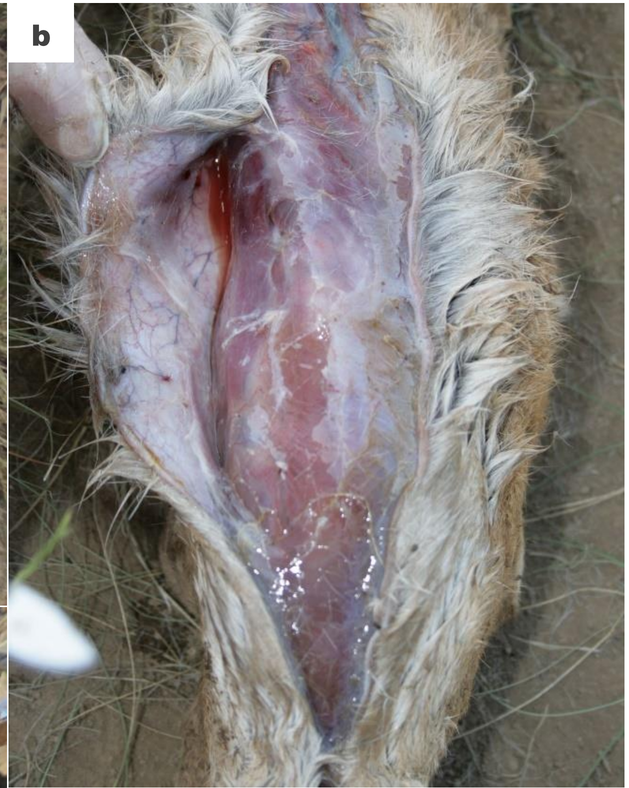

Figure 11 - Newborn saiga with death from dystocia. Note the curly fur, coated in birth fluids (a). Marked subcutaneous oedema over the head, especially beneath the mandible, extending down to the neck (b). Bruising of the jaw muscles and tongue (c).

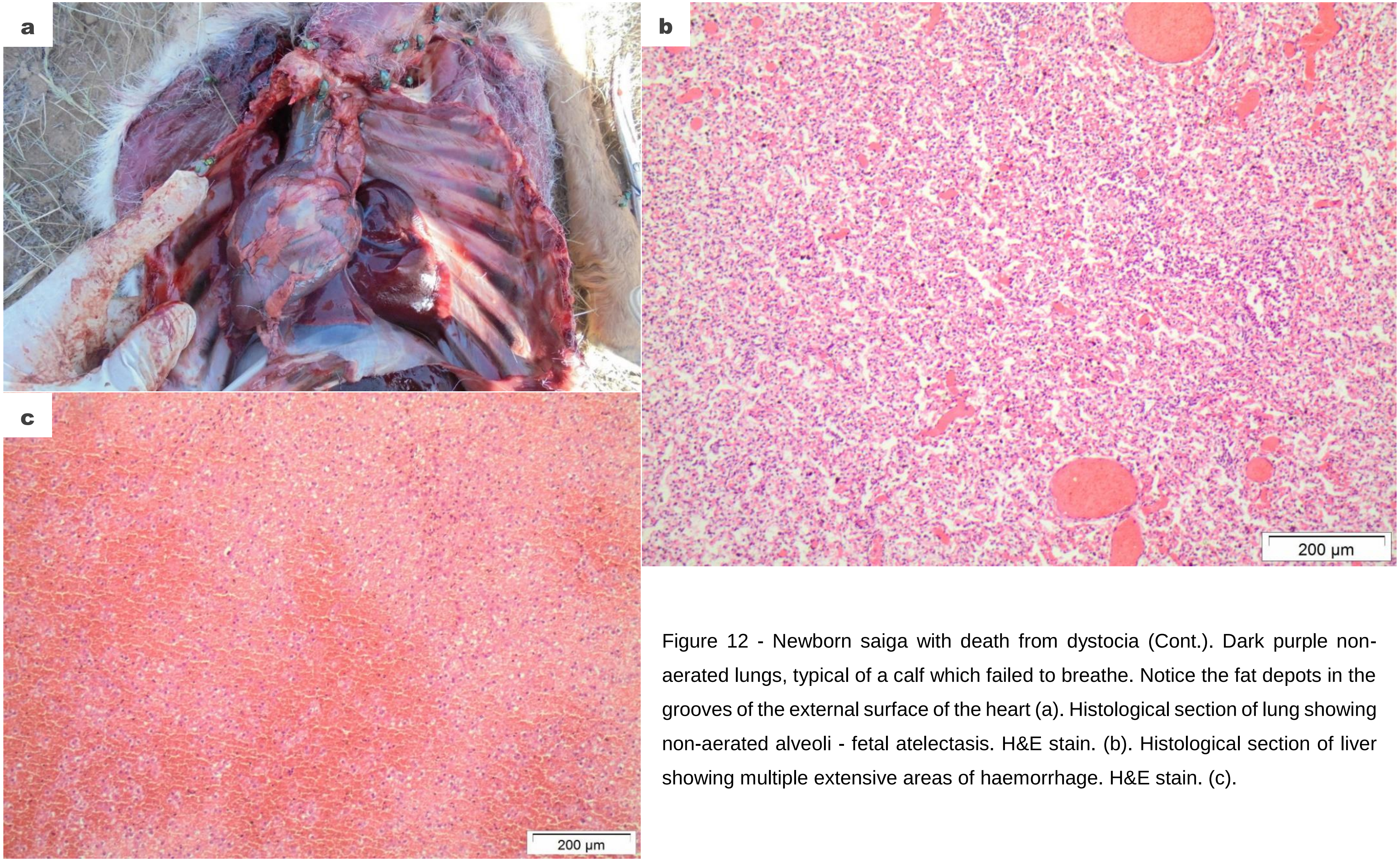

Figure 12 - Newborn saiga with death from dystocia (Cont.). Dark purple non-aerated lungs, typical of a calf which failed to breathe. Notice the fat depots in the grooves of the external surface of the heart (a). Histological section of lung showing non-aerated alveoli - fetal atelectasis. H&E stain. (b). Histological section of liver showing multiple extensive areas of haemorrhage. H&E stain. (c).

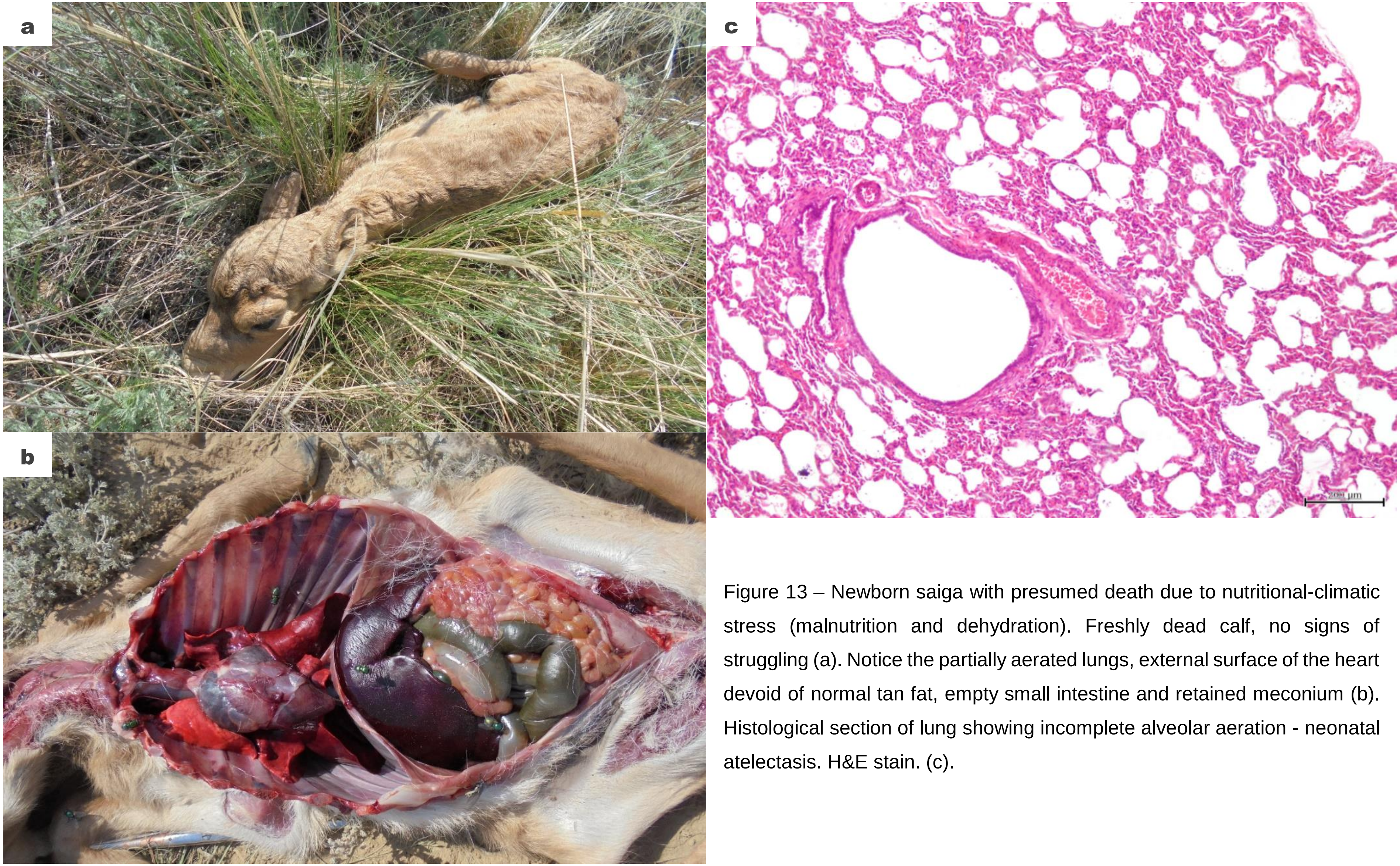

Figure 13 – Newborn saiga with presumed death due to nutritional-climatic stress (malnutrition and dehydration). Freshly dead calf, no signs of struggling (a). Notice the partially aerated lungs, external surface of the heart devoid of normal tan fat, empty small intestine and retained meconium (b). Histological section of lung showing incomplete alveolar aeration - neonatal atelectasis. H&E stain. (c).

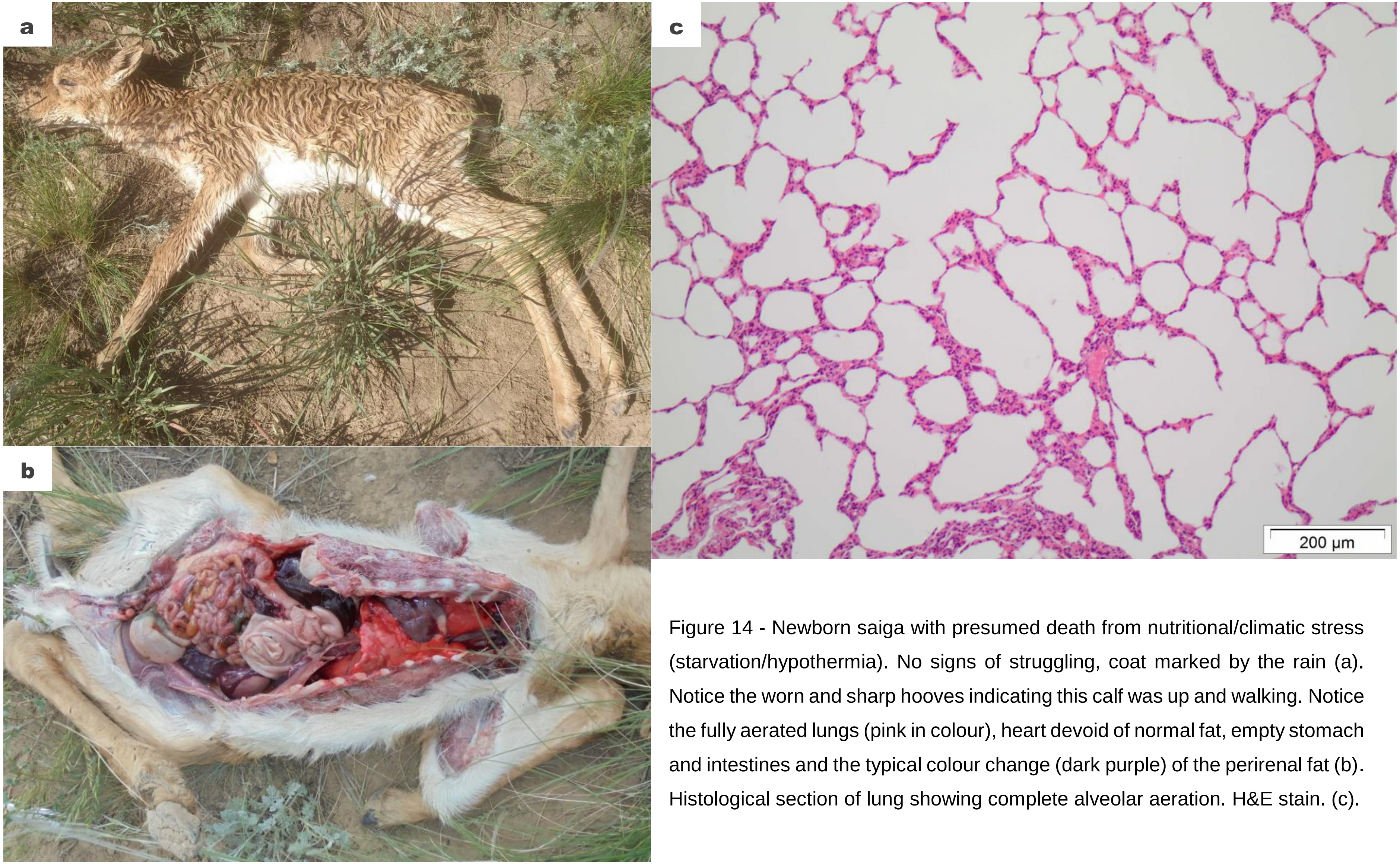

Figure 14 - Newborn saiga with presumed death from nutritional/climatic stress (starvation/hypothermia). No signs of struggling, coat marked by the rain (a). Notice the worn and sharp hooves indicating this calf was up and walking. Notice the fully aerated lungs (pink in colour), heart devoid of normal fat, empty stomach and intestines and the typical colour change (dark purple) of the perirenal fat (b). Histological section of lung showing complete alveolar aeration. H&E stain. (c).

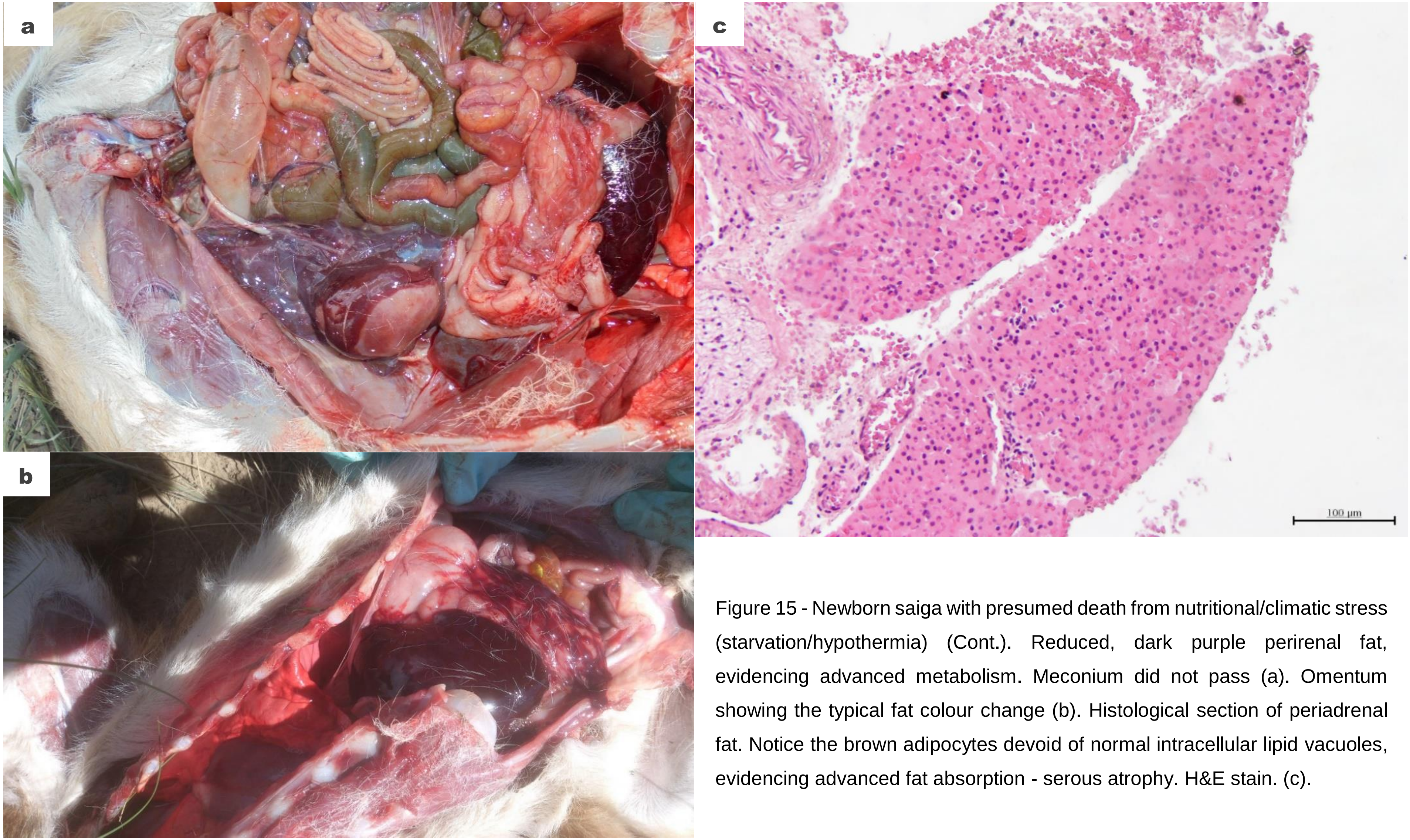

Figure 15 - Newborn saiga with presumed death from nutritional/climatic stress (starvation/hypothermia) (Cont.). Reduced, dark purple perirenal fat, evidencing advanced metabolism. Meconium did not pass (a). Omentum showing the typical fat colour change (b). Histological section of periadrenal fat. Notice the brown adipocytes devoid of normal intracellular lipid vacuoles, evidencing advanced fat absorption - serous atrophy. H&E stain. (c).

4.9 BODY WEIGHTS IN DIFFERENT CAUSE-OF-DEATH CATEGORIES

The results of the analysis of the body weights at post-mortem examination of saiga calves with different suggested cause of death are presented in Table 8. The average weight of each group of dead calves was compared to that of live calves monitored in this study (3.31 ± 0.01 kg, n=941). The mean body weight of the stillborn calves was not significantly below the mean body weight of the calves captured alive . The mean body weight of calves that died from dystocia was significantly higher (p<0.001) than that of calves in other dead-calf groups and higher when compared to live calves, although not significantly. Within the dystocia group, the mean weight of female calves (3.71 ± 0.09 kg) was higher than male calves (3.46 ± 0.34 kg), although not significantly. Dead females had a mean body weight significantly higher (p<0.05) than live calves (regardless of gender), while dead male calves were not significantly heavier, even though the largest calf deceased from dystocia was male, weighing 4.08 kg. The body mass of calves which died inside their mothers due to difficult calving was significantly lower (2.90 ± 0.17 kg, p<0.05) than that of the cases above. As previously described, calves in this group were found maldisposed in the birth canal, but apparently were not oversized or disproportioned in relation to mothers.

Table 8 - Mean post-mortem weights (± SE) in kg of saiga calves in different cause of death categories (n=35).

Categories	N	Mean	Min	Max
Stillbirth [1]	5	2.79 ±0.19	2.36	3.30
Dystocia	6	3.58 ±0.17	2.92	4.08
Malnutrition/dehydration	7	2.44 ±0.06	2.28	2.66
Starvation/hypothermia	9	2.69 ±0.13	2.13	3.22
Underweight/ underdeveloped	3	1.36 ±0.12	1.20	1.60
Mother's death with calf inside (dystocia-related) [2]	3	2.90 ±0.17	2.58	3.18
Inconclusive/unknown [2]	2	2.73 ±0.15	2.58	2.88

(1) Recording of body weight missing for one calf. (2) Recording of body weight missing for two calves.

Three dead calves were found extremely underweight, with mean body mass significantly lower (p<0.01) than that of live calves monitored during this calving period, and also compared to calves from any other dead-calf groups. The mean post-mortem weight of the newborn calves presumably dead from dehydration and exhaustion at postpartum, was significantly lower (p<0.01) than that of live calves. Similarly, calves that died due to starvation/hypothermia were significantly lighter compared to the calves captured alive.

4.10 ADDITIONAL FINDINGS OF SAIGA CALVES

In addition to the forty-seven dead calves described in the previous sections, a further twenty-three cases were reported by Team 2, in the period of May 12th to 18th. Animals were divided into different categories, as shown in Table 9, based on the information of external observations and photographic records kindly provided by this group.

Two mummified fetuses (8.7% of all), characterized by marked dehydration of the body tissues (shrunken and dry fetuses), were reported. One dead calf (4.4% of calf losses) showed mandibular brachygnathia ("parrot mouth"). A couple of cases of skeletal malformations were also observed during newborn calves monitoring. Table 10 below summarizes all cases of external malformations observed in Betpak-Dala in May 2014.

Table 9 - Saiga calf losses and sex recorded by Team 2 in the Betpak-Dala population in May 2014 (n=23).

Categories	N	%	Male	Female	Unsexed
Fetal death and mummification	2	8.7			2
Malformations	1	4.4	1		
Dystocia	7	30.4			7
Not possible to categorize	13	56.5	5	8	
Total	23	100	6	8	9

Seven calves (30.4% of calf losses) were considered as having died in the birth canal of females which failed to give birth.

Although about half of the animals reported by this team (56.5% of all, Table 9) were not strictly categorized by the author due to lack of information, seven of these (30.4% of all losses) apparently died before or not long after birth, as suggested by the presence of maternal fluids on the coat or fetal membranes attached, absence of hoof wear and/or the presence of a fresh umbilical cord. Abnormal positions were also registered for some of these calves. The remaining six animals in this group (26.1% of all losses) were most likely newborn calves that died later postpartum from unknown reasons, predation excluded.

Table 10 - Skeletal malformations in saiga calves of the Betpak-Dala Population recorded in May 2014.

Malformations	Dead	Alive
Mandibular brachygnatia	1	
Bilateral hind limb adactylia		1
Cleft palate		1
Total	1	2

4.11 TRANSECT COUNTS AND DENSITY OF DEAD ADULTS

Only two dead adult females were recorded on transects walked through the study area, corresponding to an average weighted density of 0.005 (95%, CI 0.003 - 0.007) carcasses per ha. Considering the area of interest (13144 ha), a total of 70 deaths (95%, CI 46 - 94) was estimated.

4.12 PROXIMATE CAUSES OF DEATH OF ADULT SAIGA

A total of twenty-four dead females was recorded in the period of May 12-21, 2014, in the main calving area of the Betpak-Dala population, including not only observations on transects, but throughout the area, by both teams. The post-mortem observations and respective suggested causes of death are presented as follows.

Dystocia occurred in ten of the 24 dead females (41.6%) found in the study area (Table 11). Evidence of struggling around the body area and fetus (or fetal membranes) present at the vulva could be observed in all cases. In five females, fetuses were in anterior presentation with abnormal disposition of head and/or front limb and one case of simultaneous presence of two fetuses in the birth canal was identified (Figures 16 and 17). In the remaining cases, the available data was insufficient to determine the reason for dystocia. Four of the females, those which were examined by the author, were estimated at ≥ 3 years of age (Table 12). Information on age was not obtained for the cases reported by Team 2.

Uterine prolapse, in most cases visible as a large mass of tissue with prominent caruncles protruding from the vulva, was recorded in about 29% of all cases (Table 11) and occurred in females of any age group (Table 12).

Table 11 - Causes of death assigned to adult saiga females of the Betpak-Dala population at calving 2014 (n=24).

Cause of death	N	%
Dystocia	10	41.6
Uterine prolapse	7	29.2
Parturition-related trauma	1	4.2
Predation	2	8.3
Undetermined	4	16.7
Total	24	100

The death of one of the examined animals (4.2% of all cases) was attributed to parturition-related trauma (Table 11). The animal was found alive, alert and active, making repeated attempts to rise and walk. Physical examination revealed trauma of the birth canal, with perineal damage (swollen perineal area/torn and bloody vulva) and partial hind limb paralysis. No changes were detected in the forelimbs. On palpation, crepitation within the right hip joint was noted. Full post-mortem examination revealed good body condition, as indicated by plentiful fat stores, and complete hip fracture at the level of the head of the right femur, with associated haemorrhage. Bruising of the uterine body and vagina were observed, suggesting difficult parturition. Extensive histopathological examination was carried out without significant changes detected. Microbiology and biochemistry exams results were not available. Primary cause of death was attributed to dystocia-related nerve injury (probably obturator), loss of function of the adductor musculature and secondary trauma to the pelvis and hip from the resulting spread-eagled gait. This would most probably be due to fetal-pelvic disproportion, though not confirmed. A similar clinical presentation was observed in another young female, which did not succumb during the study period and, therefore, was not examined post-mortem. Two deaths (8.3% of all losses) were attributed to attack by a predator (Table 11). Both females were pregnant when attacked and showed bite wounds on different parts of the body. One of the females showed penetrating wounds with purulent exudate on the abdomen, bruising and haemorrhage of the cervical muscle layers, penetrating injury of the cervical trachea, swelling around the larynx and presence of large amounts of blood in the lungs. A single fetus was normally engaged in the birth canal, but the membranes had not yet ruptured and the cervix was not fully open. The other female also showed penetrating wounds on the neck and severe perineal trauma. One fetus was removed from the uterus and was entirely covered with meconium, which is suggestive of intrauterine stress. Apparently a second fetus was missing. Predators involved in these attacks were most likely wolves, possibly wild boar (suspected by local experts in the first case), or less likely foxes.

In 16.7% of the cases, the primary cause of death was not possible to determine (Table 11). One of the females was found in advanced state of decomposition, so that the cause of death could not be identified. The group also includes two females reported by Team 2, on which the data available were not sufficient to assign a cause of death, thought predation was ruled out. Another case was one mature female, in postpartum condition, that showed extensive bruising of the muscles over the thorax, suggesting trauma of unknown origin. Post-mortem examination revealed lungs diffusely red, with frothy fluid exuding from the bronchi, with no other recognizable primary lesions found. Extensive bacteriological and histopathological examinations were carried out by laboratory technicians present in the field and the author respectively. The main histological feature was a striking pulmonary oedema. A discrete inflammatory component, with infiltration of interalveolar septa by mononuclear cells, was also observed. No other significant changes were detected histologically. Microbiology results were not available for evaluation in this study.

Table 12 - Estimated age of 14 saiga females examined in the Betpak-Dala population, in May 2014, by visual assessment (eruption and tooth wear, general appearance of the animal). Given that all animals are born in May, they are all of integer age. The individuals were classified as 1 year-old, 2 years-old and ≥ 3 years-old.

Cause of death	N	1 year	2 years	≥ 3 years
Dystocia	4			4
Uterine prolapse	5	2	1	2
Parturition-related trauma	1		1	
Predation	2			2
Undetermined	2			2
Total	14	2	2	10

4.13 INCIDENTAL PATHOLOGICAL FINDINGS IN ADULT SAIGA

Necrotizing hemorrhagic cystitis was observed in three females which died as a result of reproductive disorders, including dystocia, uterine prolapse and parturition-related trauma (Figure 18). A chronic inflammatory nodular lesion in the liver, comprising a central area of necrosis partially calcified, surrounded by fibrous tissue, was an incidental finding in one female which died from attack by a predator. The causative agent (possibly parasitic) was not determined. This female also showed chronic multifocal pleuropneumonia, characterized by lesions of chronic interstitial pneumonia with fibrous thickening of the interalveolar septa, and focal pulmonary osseous metaplasia.

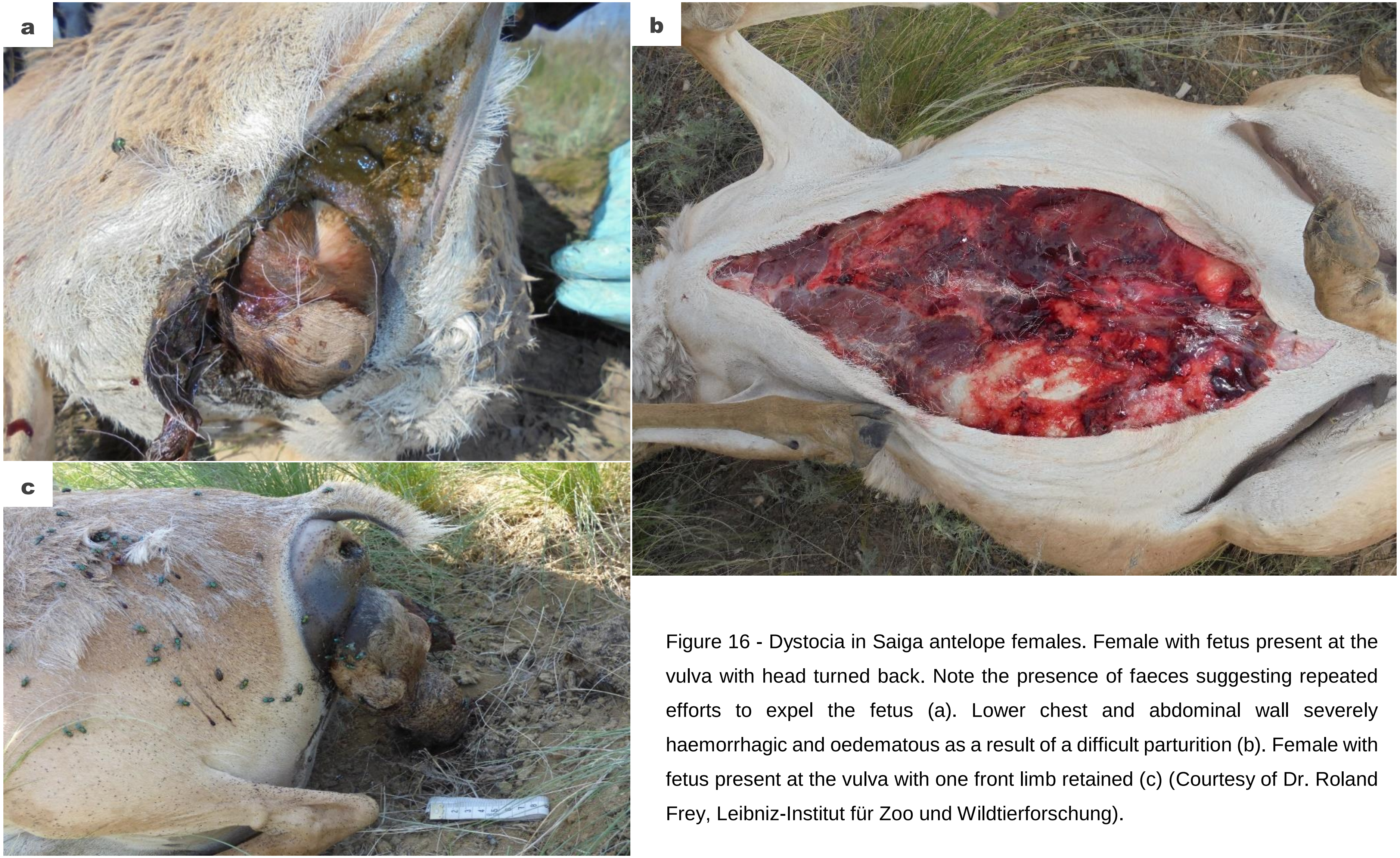

Figure 16 - Dystocia in Saiga antelope females. Female with fetus present at the vulva with head turned back. Note the presence of faeces suggesting repeated efforts to expel the fetus (a). Lower chest and abdominal wall severely haemorrhagic and oedematous as a result of a difficult parturition (b). Female with fetus present at the vulva with one front limb retained (c) (Courtesy of Dr. Roland Frey, Leibniz-Institut für Zoo und Wildtierforschung).

Figure 17 - Dystocia in Saiga antelope females (Cont.). Female with fetus present at the vulva with the head turned back. Notice evidence of struggling around the body area (a). Female with simultaneous presence of twins in the birth canal (b) (Courtesy of Dr. Roland Frey, Leibniz-Institut für Zoo und Wildtierforschung).

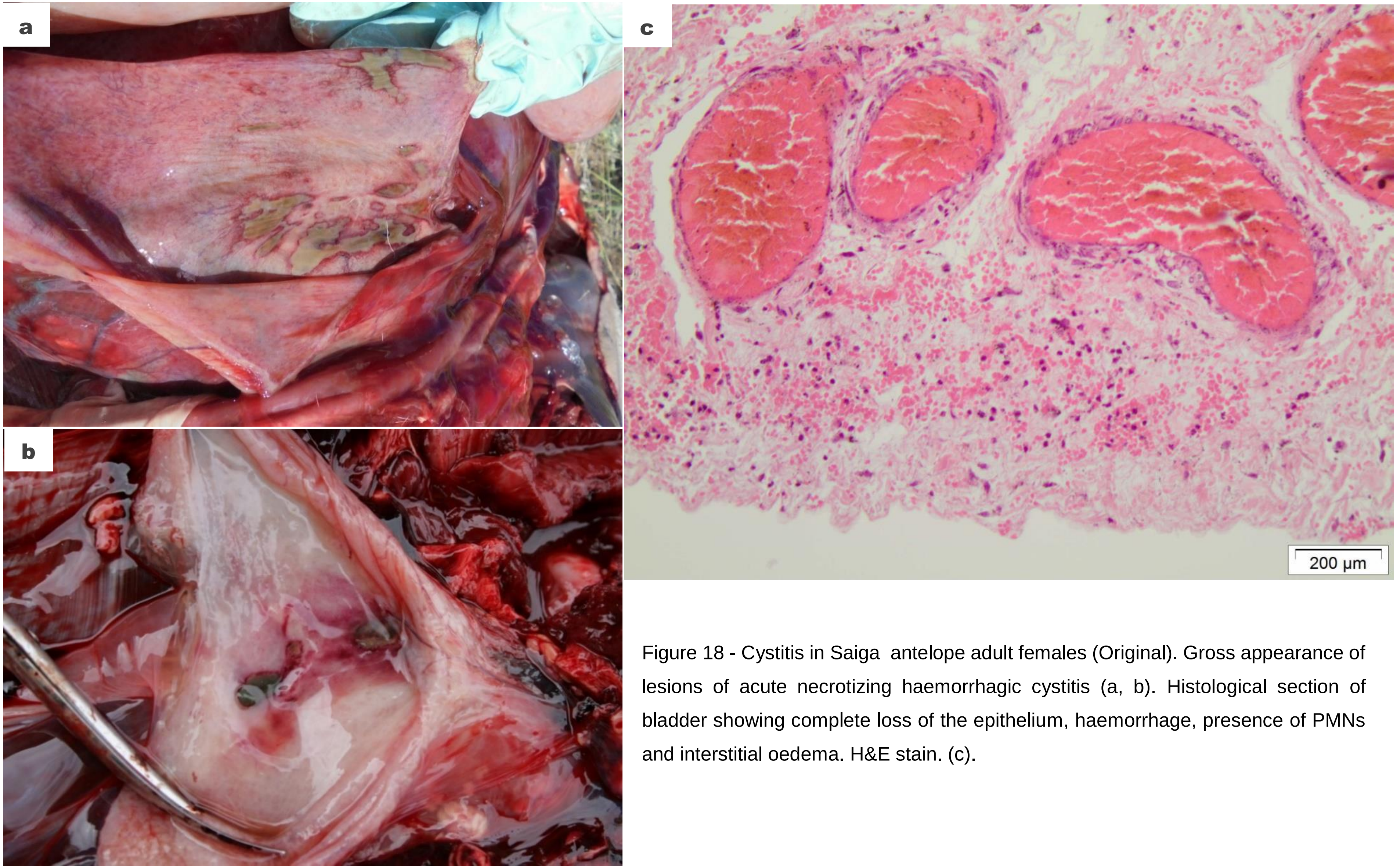

Figure 18 - Cystitis in Saiga antelope adult females (Original). Gross appearance of lesions of acute necrotizing haemorrhagic cystitis (a, b). Histological section of bladder showing complete loss of the epithelium, haemorrhage, presence of PMNs and interstitial oedema. H&E stain. (c).

5 DISCUSSION

5.1 SEX RATIO AT BIRTH

The results of this study suggest a slight predominance of males among newborn saiga in the Betpak-Dala population in 2014. These findings are close to those of investigations conducted by ACBK in 2012 and 2013 in the same population. At that time, the sex ratio among newborn saiga was 1:0.87 (n=713) and 1:0.89 (n=733) respectively (ACBK's unpublished data, 2014). The methods of sampling and capture used in the different surveys were similar and the results, therefore, comparable. According to the available literature, the sex ratio of saiga at birth is generally close to 1:1, with slight fluctuations from year to year (Fadeev & Sludskiy, 1982; Bekenov et al., 1998). Lundervold (2001) found a significant difference between the sex ratio recorded in the Betpak-Dala population in 1997 (in favour of newborn females 56.2%) and the Ustiurt population in 1998 (in favour of newborn males 55.1%). Lundervold's capture methods in both populations were identical, suggesting that the differences were not caused by a sex bias in capture. Table 13 summarizes the available data on long-term sex ratios of neonates for the 1960-1970s, 1980s and the period 2012-2014, which seem fairly consistent. Fadeev and Sludskiy (1982) suggested that the slight differences in sex ratio are possibly related to differences in population size, since these authors noticed that in years when saiga population in Kazakhstan was at its peak (in 1972 and 1974), sex ratio of embryos was in favour of males (1:0.8), and when it was at its lowest (period 1976-1978), more females were recorded (1:1.5 to 1:2). Bekenov et al. (1998), in contrast, on analysis of data from 1983 to 1993, did not find a clear relation between the slight fluctuations observed in the newborn sex ratio and population size or climatic conditions. Explanations for these differences in sex ratio among newborn saiga between years and populations require further investigations.

Table 13 - Sex ratio of newborn saiga . Comparison of data in the 1960-70s (Fadeev & Sludskiy, 1982), 1980s (Bekenov et al., 1998) and 2010s (ACBK's data and our observations).

Period	Sample size	Males (%)	Females (%)	Ratio
1960-70s	18 781	51.8	48.2	1:0.9
1980s	17 114	51.2	48.8	1:1
2012-2013	2302	53.6	46.4	1:0.9

5.2 BODY MASS OF SAIGA CALVES

Newborn calves in the Betpak-Dala population weighed on average 3.31 kg (SD 0.40) in 2014. This value is lower compared to the mean weight reported for the same population (3.48 kg, SD 0.45) in 1997 and similar to that obtained for a sample of the Ustiurt population (3.30 kg, SD 0.41) in 1998 (Lundervold, 2001). Differences in the body mass of offspring in different years and populations have been reported in Saiga antelope (Kühl, 2008; Bayarbaatar, 2011) and are likely related to changing environmental conditions (Bekenov et al., 1998; Coulson et al., 2000; Bayarbaatar, 2011). However, it should be noted that methodologies in weighing (e.g. less accurate scales) as well as in the sampling (for instance, capture methods allowing sampling of older and, therefore, heavier animals) may interfere with results if proper interpretation is not made. Male calves were found significantly heavier than females (240 g difference on average). We assume that this is not an effect of considerable differences in age between males and females, given the large sample size investigated. These results are in line with previous studies suggesting that saiga mothers allocate more resources to male than female offspring (Bannikov et al*.*, 1963; Fadeev & Sludskiy, 1982; Kühl, 2008).

5.3 Calving process

5.3.1 Temporal distribution of births

Mass calving of the Betpak-Dala population in 2014 took place within a relatively short period of time in late spring, during the second 10 days of May, as commonly observed in this population (Fadeev & Sludskiy, 1982; Bekenov et al., 1998). Saiga antelopes live in highly seasonal steppe and semi-desert ecosystems (Bekenov et al., 1998; Milner-Gulland, 2001), so that calving seems to be scheduled to coincide with the most suitable period of the year, when the warmer weather is beginning, fresh green vegetation is growing and watering places are available (Bekenov et al., 1998; Zhirnov et al., 1998). Births apparently showed a modal distribution, with densities of newborn saiga rising to a peak and then declining over the following few days. The onset of calving took place around May 10 or 11, which led to a certain difficulty in capturing calves, when transects started being performed on May 12. The higher average body mass of calves captured at that date might reflect this slight difference in age. From the distribution of calf density throughout the survey period, it can be inferred that the peak of births took place within the period from May 15 to 18, since the highest densities of calves on the calving grounds as well as the highest proportion of captured calves (76% of all) occurred during this period. Very few newborn calves were found in the area on May 21, putting the end of the calving season at around this date. This is also supported by observations made the following day, when the herd, consisting mainly of adult females and calves, was located significantly further south. A similar distribution pattern was previously observed in saiga populations, according to data on the temporal aggregation from expeditions undertaken by the Institute of Zoology of the Kazakhstan Academy of Sciences in the 80-90s (Milner-Gulland, 2001).

5.3.2 Spatial distribution of births

Newborn calves were found distributed in the birth areas at an average density of 3.49 (95%, CI 2.5 - 4.49) individuals per ha. Fadeev and Sludskiy (1982) reported an average of 5 to 7 calves per ha in the same population in the past. It should be noted that the average obtained in this study includes densities measured at the very beginning and end of the calving period, when the numbers of calves in the birth places were much lower. Inclusion of these days in the calculations may have contributed for a lower estimate of the density of calves for this population in comparison to the values reported by Fadeev and Sludskiy (1982). In fact, if calculations are limited to the peak of calving period, average density rises to 6 individuals per ha, which falls within the density range reported by these authors. Saiga antelopes tend to show not only temporal synchronization of births, but also spatial clustering, which is assumed to be a biologically significant mechanism of reducing predation on vulnerable newborn calves, especially from wolves, which have a more territorial structure (Bekenov et al., 1998; Milner-Gulland, 2001). These calving aggregations were reported to be decreasing, especially in the Ustiurt population, as a result of the general declines in population size following the breakup of the Soviet Union (Milner-Gulland et al., 2001) and increased poaching and human disturbance (Kühl et al., 2009[a]), which may potentially change the effect of predators (Milner-Gulland, 2001). In 2014 in Betpak-Dala, females gathered in large numbers, but the exact size of the aggregation was not possible to determine.

5.3.3 Potential role of temperature

The results of the analysis of calf densities and air temperatures suggest that there might be a relationship between births and environmental temperature, with larger numbers of newborns being recorded when the climate was warmer. The count results made evident a modal distribution in the numbers of calves over time; if ambient temperature during the period in study is taken into account - its variance curve superimposed with the density plot - a possible role for this variable on the time of calving becomes somewhat apparent. Although the number of density samplings does not allow for the establishment of a definitive cause/effect relationship, the significant statistical trend found between densities of newborn calves and the values for ambient temperature does warrant attention regarding this matter. Parturition date is generally accepted as being mainly determined by the mating date during the rut, in itself related as well with the anticipation of greater abundance in feed and environmental conditions more favorable to calf survival (Bekenov et al., 1998; Zhirnov et al., 1998). However, the curious finding of a relationship between the number of newborn calves and temperature suggest the possibility of a more short-term influence of the environment on the birth dates. Data on local temperature and calving behaviour obtained over multiple years would be necessary to either prove or discredit such a cause/effect relationship between weather and time of calving in Saiga antelope.

5.4 MORTALITY IN NEWBORN SAIGA

5.4.1 Level of mortality

Mortality during the 2014 birthing period of the Betpak-Dala population involved mostly calves, measured as an average density of 0.1 (95% CI 0.07 - 0.12) carcasses per ha and a resulting rough estimate of 1314 deaths (95%, CI 920 - 1577) in total in the whole calving area. Based on the transect counts of dead and live calves, a proportion of 4.4% dead (95% CI 2.3 - 6.6) was further estimated. Although these point estimates and respective CI´s were obtained, the actual mortality is likely to be somewhat higher than estimated, as a consequence of the fact that, while it corresponds to a phenomenon occurring dynamically in space and time, transect sampling itself can be conceptually seen as a snapshot. Therefore, the values reported here possibly represent lower bounds for the mortality as some dead calves might have been missed for a number of reasons, including: (1) animals potentially dying at a given transect after it has been surveyed; (2) animals missed due to removal by predators or scavengers; (3) animals missed due to imperfect detectability. The repetition of transects in order to quantify later deaths was not possible due to constraints in time and staff. However, for instance, on May 19, when part of the areas surveyed on days 16 and 17 were revisited, newborn calves were hardly seen, as most had already joined their mothers and the herd, and additional carcasses were not identified within transect areas. Moreover, according to field observations, the density of scavengers and predators was apparently low in the core calving area, with only crows and steppe eagles occasionally observed nearby. Steppe eagles (*Aquila rapax*) seem, however, less likely to succeed than other birds of prey, such as golden eagles (*Aquila chrysaetos*), given their smaller size, usually feeding on rodents, such as sousliks (*Spermophilus fulvus;* Sklyarenko & Khrokov, 2008). A single fox was observed at the beginning of the expedition, but not in close proximity to the calving area, and no other predator (e.g. wolf) was ever sighted in the area, though post-mortem examination data indicates a few cases of attacks.

There is little comparable knowledge on mortality of newborn saiga (*Saiga t. tatarica*). According to data presented by Zhirnov et al. (1998), in apparently regular calving seasons of the Kalmykia population, at distinct places, 51 deaths among 544 calves (9.4%) were recorded in 1957 and 70 among 874 calves (8%) in the following year. In 1959, in the same population, out of a total of 1238 calves, 64 were found dead (5.2%), despite it being a drought year. It is mentioned that the actual mortality rates were likely to be higher, with some animals possibly missed due to predation. Specific details on the methodology used in these investigations are not known. The estimates for the Betpak-Dala population in 2014 are lower compared to these references. Bekenov et al. (1998), citing Grachev & Bekenov (1993), reported a calf mortality at birth or during the first days of 0.2-6.8% in the period 1989-1993, with up to 50% of deaths attributed to human factors. A reasonable comparison with these results is not possible, given that little information is provided by the authors, but the levels reported, although close to this study, seem very much differing from year to year and surprisingly low, at a time when human disturbance was high due to a greater proximity between the saiga and human populations. Another available reference is a study by Bayarbaatar (2011), in the Mongolian population (*Saiga t. mongolica*), in which the survival rate ±SE (in 116 radio-collared calves) in the calving period was 0.85 ± 0.06 in 2008 (n=40), 0.80 ± 0.06 in 2009 (n=40) and 0.89 ± 0.05 in 2010 (n=36). However, mortality in Bayarbataar's study refers to a period ranging as far as 12-14 days from the capture date, far longer than that of our study, in which mortality was recorded during the first few days of life. Furthermore, Bayarbataar's study points to a significant abundance of predators (raptors and red foxes) in the area, with predation being not only observed on the marked animals, but also on unmarked animals, and cases of predation on neonates being frequently witnessed by the researchers. As discussed above, the same was not true for the calving area scanned in this study.

5.4.2 Effects of sex

Interestingly, more female calves were found dead than males (ratio 2:1). Considering that sex ratio at birth was slightly in favour of males, the opposite could be expected for the simple reason that more male newborns were present in the area. As newborn females generally showed lower body weight compared to newborn males, and, additionally, dead calves had an average body mass significantly below that of newborn calves monitored alive, the birth weight likely played a role in newborn survival, perhaps explaining the sex-differential mortality observed. Bayarbaatar (2011) had already reported survival rates of male and single calves consistently higher in different seasons (including calving) than those of female and twin calves. Additionally, male single calves weighed more than female and twin calves individually, supporting the idea that calves heavier at birth have higher survival rates than lighter calves. Despite the differing approach to sampling taken in this study and the small sample size investigated (n=33), current findings seem in line with those of this researcher.

5.4.3 Effects of weather

Calving is timed to coincide with the most favorable period of the year, when the unstable spring weather is at its end and temperature is getting higher. The sporadic occurrence of inclement weather at this time (cold rain, hail, frosts) has been associated with increased mortality among saiga newborns (Fadeev & Sludskiy, 1982; Bekenov et al., 1998; Zhirnov et al., 1998). For instance, in Kazakhstan, in 1966, 1969, 1970, 1971, and 1975, temperatures reached -3 to -7°C, resulting in the death of 30 to 44 calves from cold during the calving period (Fadeev & Sludskiy 1982). In the current study, potentially adverse weather conditions were observed in the field over the end of the survey period, raising the question of a possible effect of weather on calf mortality. Based on the statistical analysis made, no significant relationship between densities of dead calves and air temperatures could be established. However, this might be due to insufficient density measures obtained from May 19 onward, when temperatures declined. Regardless, despite the shortage of data, the higher densities found later in the study - about 0.2 carcasses per ha (20 carcasses/km^2), on average - do suggest a causal role of weather conditions in mortality, a fact supported by field observations of an increased number of carcasses located outside the transects. The results showed a continued increase in the densities of dead calves over time. However, the initial increase in absolute numbers of dead calves is more likely due to changes in density of the calf population in the calving area at the time of survey (i.e., with greater numbers of animals born, likelihood of finding dead animals increases), and may not reflect a direct increase in mortality. On the contrary, as the survey period progressed, maximum density of calves born was achieved and the number of dead calves would therefore be expected to settle, instead of rising toward the end of the calving period. This may have been due to the onset of the period of lower environmental temperatures, which coincided with this later period of the survey, as is in part confirmed by the greater number of deceased calves found during this period with changes compatible with starvation/hypothermia. It should be considered, however, that the majority of births seemingly took place under warmer weather conditions, prior to the observed weather changes, which may have contributed to mitigate mortality, since less animals were born under cold and windy weather. Therefore, it might be that a drop in temperature at the latter stage, when animals were more active and benefiting from metabolic heat, might have had a minor effect on mortality.

5.4.4 Proximate causes of death

Little knowledge on cause-specific mortality of saiga calves is documented. Nutritional and environmental stress were the major causes of saiga calf mortality (34% of the cases) in the Betpak-Dala population during the 2014 calving season. Calves included in this category were generally found in poor nutritional condition, showing varying degrees of dehydration and body fat completely or partially depleted. In addition, none had presence of milk in the digestive tract. These constitute significant findings, as young ruminants are yet to develop the capacity to digest fibre at this age, depending exclusively on maternal milk for their nutrition (Porter, 1969). If they fail to feed they immediately begin to metabolize their body fat depots (Mc Farlane, 1965). Several factors predispose neonates to death from nutritional stress within the first days of life, including fetal malnutrition with poor fat reserves at birth, postpartum hyponutrition and increased energy demands due to cold exposure (Brown, Baker, & Barker, 2007). From the observations made, some of the dead newborns, presenting with rare signs of hoof wear and apparent incomplete lung expansion, were deemed too weak after birth to be able to stand and suckle. Prolonged parturition, perhaps of twins or triplets, and/or a low birth mass may also have played a role, possibly resulting in calves with poor vigour at birth, weak respiratory movements and inhibited behaviour in suckling. Factors on the mother's side, such as maternal death or neglect, could not be ruled out. For instance, two adult females were found ill/dead in the proximity of emaciated calves which could well be their offspring. Maternal neglect is very difficult to confirm in free-ranging saiga herds but it was reported in captivity (Dolan, 1987; Pohl, 1987) associated with birth complications, especially with multiple births (Pohl, 1987), and suggested in the wild, with calves being possibly abandoned when the herd is disturbed and disperses (Bekenov et al., 1998). Calves may also simply lose their young and inexperienced mothers among thousands of other calves in the birth aggregations (Bannikov, 1963). Neonates that lose their mothers are unlikely to survive, unless they are raised by an adoptive mother, as previously observed in mothers who had stillbirths. Older calves, in turn, may be able to suckle from other mothers, if they are strong and persistent enough (Pohl, 1987). The findings in this study suggest that adverse weather conditions (cold temperatures, rainfall and wind) likely played a role in predisposing saiga calves to death from a proximate cause to hypothermia or starvation. However, the effect of these climatic stresses would probably have been more significant if they had occurred during the birthing peak, with neonates in the first hours of life being particularly vulnerable to death from hypothermia or inability to suckle (McCutcheon, Holmes, & Mcdonald, 1981; Thompson, 2003). Thus, in many cases, the inclement weather might have simply accelerated the pending deaths of already malnourished and starving calves, which are likely to be more susceptible to hypothermia than calves which are well fed (McCutcheon et al., 1981). For logistical and legal issues, specifically related to international transport of biological samples, microbiological sampling and analysis were not carried out from any of examined animals. Although microbiological results are not available in this study, the lack of infection-related pathological findings at post-mortem examination supports the idea that infectious conditions were unlikely involved and mortality was mainly attributable to nutritional deprivation of calves as well as environmental stress.

Dystocia was the second most important cause of calf mortality (23.4%), included the cases of calves found dead inside females which failed to give birth. Since mothers were not always examined internally, and saiga often produce twins, the true number of fetuses is likely to be higher. Additional data from Team 2 also showed dystocia as a significant source of mortality, with 70% of calf classifiable deaths attributed to this factor. In addition to the cases categorized as dystocia in this study, difficulties in the parturition could have also preceded other postpartum events, such as deaths due to nutritional or climatic stress. It has been well documented that lambs who face difficult births may have trouble maintaining body temperature as well as show inhibited behaviours in teat searching and suckling (McCutcheon et al., 1981). Dystocia occurs as a result of a number of complex factors, either maternal or fetal, relatively well studied in domestic ruminants. These include systemic conditions (e.g. maternal peripartum hypocalcaemia), incomplete cervical dilation, pelvic malformations, abnormal presentation, position or posture of the fetus and fetal-pelvic disproportion (Hindson

& Winter, 2007; Edmondson, Roberts, Baird, Bychawski, & Pugh, 2012). The findings of this study suggest that birth weight in a few cases may have contributed to dystocia (perhaps in primiparae), with larger female calves (body weights above average in 2014) more often involved than males, though this may be an artifact caused by the small sample size. In domestic ruminants, birth weight has been reported as a leading risk factor to dystocia in singletons (Fogarty, 1992). However, fetal maldisposition/malpresentation were often observed in calves which died in the mother's birth canal, suggesting that this is a common feature of dystocia in Saiga antelope, as will be discussed further ahead.

A total of 12.8% in calf mortality was attributed to stillbirths in the 2014 calving season. Based on the criteria used, none of the calves was viable after birth, as they failed to breathe (non-aerated lungs), though death could have occurred before (i.e. intrauterine death), during or immediately after birth (i.e. parturition stillbirth). For instance, three calves were found relatively well preserved, suggesting that their death did not occur until very close to parturition. Advanced autolysis or mummification would be expected in calves dead for a long time before the onset of the birth process (Mc Farlane, 1965). One of these calves had its head fully covered by the amniotic membrane, which might suggest a death associated with airway obstruction. In contrast, the other three stillborn calves were found decomposed - placentae attached to one of them - and, thus, antepartum death could not be ruled out. However, considering their normal body weight for a newborn saiga , the causes of death are unlikely to have occurred until very late in gestation. The occurrence of stillbirths was previously reported in Saiga antelope both in the wild (Bannikov, 1963; Fadeev & Sludskiy, 1982; Bekenov et al., 1998) and in captivity (Dolan, 1987; Pohl, 1987). At Tierpark Berlin, stillbirths occurred in 12 of 116 calves (10%), involving mostly single calves of primiparae, but the exact causes were not determined (Pohl, 1987). In the wild, adverse environmental conditions, such as droughts and disturbance during calving, were associated with increased incidence of stillbirths (Fadeev & Sludskiy, 1982; Bekenov et al., 1998).

The frequency of calf mortality due to predation was low in the current study. As previously stated, these results are in contrast to those of an earlier investigation conducted in Western Mongolia, in which during the calving seasons of 2008-2010, 81% of deaths in calves were attributed to predation (raptors, foxes or lynx) and the remaining 19% to unknown causes (Bayarbaatar, 2011). It is interesting to note that no cases of malnutrition or starvation were reported by this author, unlike the present study, in which this constituted an important cause of mortality. Since ill or weak animals are easy prey for predators, in populations under high abundance of predators, malnutrition or starvation in calves are likely less common. This is in agreement with previous works, in which nutritional conditions and adverse weather were reported to be common causes of death in young wild ungulates under low predation pressure (e.g. Andersen & Linnell, 1998). Moreover, It should be noted that Bayarbaatar's method of monitoring involved radio collaring 1 to 3 days-old calves, which, for obvious reasons, excludes stillbirths and reduces the chance of including animals deceased at the first few hours after birth in his roll of causes of death in saiga calves. This results in a bias of Bayarbataar's sample towards vigorous new-borns.

Little is known about the occurrence of congenital malformations in Saiga antelopes. A congenital heart defect (patent ductus arteriosus) was found in one calf. In domestic ruminants, ductal functional closure usually occurs within the first hours after birth (Maxie & Robinson, 2007) and normal ruminants rarely have a patent ductus arteriosus after birth (Cook, 2003). This seems to support the idea that the animal in question was most likely premature at birth, a fact supported by its extremely small size and low body weight. Externally visible malformations were reported in three (below 1%) of the calves sampled on the transects (n=856). Skeletal abnormalities of the oral cavity (brachygnathia and cleft palate) and limbs (adactylia), as observed in saiga , have been previously described in other ruminants, both domestic and wild (Thompson, 2008; Hoy, Haas, Hoy, & Hallock, 2011).

5.5 MORTALITY IN ADULT SAIGA

5.5.1 Level of mortality

Adult mortality during the 2014 calving season was low, measured as an average density of 0.005 carcasses per ha and a respective total estimate of 70 deaths (95% CI 46 - 94) in the calving area. It is unlikely that these values have been significantly underestimated by the sampling scheme. Considering that the deaths were mainly related to parturition, with most females apparently dying while giving birth or shortly after, and that the daily transects were walked when the births had already occurred, it is quite likely that nearly the total of carcasses was found in the transect areas. Furthermore, the detection of adult carcasses is easier compared to calves, given their larger body size. There are few available data on the background mortality of adult saiga at calving. Fadeev and Sludskiy (1982) reported an average density of 0.009 females per ha, ranging from 0.001 to 0.01 carcasses per ha in a normal calving season, which seems to be in line with our findings for 2014. Details on the study population or the sampling method used are not provided, preventing a more appropriate comparison.

In addition, it should be pointed out that no adult males were found dead in the aggregation area. According to the ecology of this species, the birth aggregations occurring during the spring migration consist mainly of females as the majority of saiga males continue the migration ahead to the summer pastures (Fadeev & Sludskiy, 1982; Bekenov, et al 1998). Therefore, adult male mortality is not an expected finding for this period under normal circumstances and should warrant a further investigation.

5.5.2 Proximate causes of death

Problems related to parturition, including dystocia, uterine prolapse and trauma, accounted for the death of 18 of 24 saiga adult females (75%).

Dystocia was the most frequent condition found in adult saiga during the birthing period monitored (41.6% of all cases) and led to death both mother and offspring. Dystocia has been documented for a number of wild ungulates, including wild elk (Lehman, Schmitz, Rumble, Kragel, & Millspaugh, 2012), wild caribou (Bebgerud, 1971), pronghorn antelope (Dunbar, Velarde, Gregg, & Bray, 1999; Jacques, Jenks, Sievers, Roddy, & D.E., 2007), roe deer (Zele, Bidovec, & Vngust, 2006), moose (Testa, 2004) and musk oxen (Norment, 1980). In this study, postural abnormalities of head and/or forelimbs of the fetus and simultaneous presentation of twins were identified as etiological factors of dystocia in 6 of the 10 cases observed (60%), and could not be ruled out in the remaining 4 cases. There is no prior knowledge regarding reproductive disorders in Saiga antelopes. In caprine and ovine, malpresentation or incorrect postures are the most common types of dystocia (Hindson & Winter, 2007; Edmondson et al., 2012). Findings in this study suggest the same trend in Saiga antelopes, although a proper examination of all the females would have given a more complete picture and enable much firmer conclusions. Saiga show frequent twinning (and, more rarely, triplet births) from the age of 2-3 years onward (Fadeev & Sludskiy, 1982; Bekenov et al*.,* 2001; Kühl et al*.,* 2009[b]). This may be an important predisposing factor to the occurrence of dystocia amongst mature animals. Generally, females are sexually mature from 7 months of age and most of them produce a single calf in their first year (early reproductive maturity). Mothers are also known to invest heavily in their offspring (Kühl, 2008). With these reproductive features, saiga females (especially yearlings) may also be prone to dystocia associated to fetal-pelvic disproportion, either due to an immature pelvis, an unusually large calf or both. Two young females were found with hind limb paralysis presumably secondary to parturition trauma, in which dystocia due to fetal-pelvic disproportion may have very possibly occurred, although this could not be confirmed.

Uterine prolapse was the second most frequent disorder observed (29.2% of all losses), affecting both primiparous and mature mothers. To the best of the author's knowledge, mortality attributed to uterine prolapse has not been documented in Saiga antelope prior to this study. Prolapse of the uterus commonly occurs in ruminants after parturition. Factors causing uterine hypotony, such as prolonged dystocia, postparturient hypocalcemia and ingestion of estrogenic plants have been associated with the occurrence of uterine prolapse in cows (Foster, 2007). It is possible that the same sort of influences operate in wild ruminant species, including saiga , but very little knowledge has been documented.

Adult mortality attributed to predation during calving period was found to be negligible in this study, with only two cases identified. Both females were in unfavorable conditions, having possibly been attacked while giving birth or very close to the delivery. As suggested by the bites and injuries, wolf was the most likely predator. A possible attack by wild boar was also suggested, although cases of wild boar preying on Saiga antelope have never been reported before. Interestingly, these deaths occurred at the end of the survey period, when presumably fewer births took place. Giving birth later and solitarily might have played a role in making these females more vulnerable to predation.

6 CONCLUSIONS

Our study reports patterns of mortality of Saiga antelope during a regular calving season, in contrast to outbreak years reported recently. Mortality during the 2014 calving season affected mostly newborn calves and was primarily attributed to nutritional deprivation, climatic stress and difficult birth. During this calving period, apparently, predation was not a significant source of mortality. The results also reveal a higher frequency of deaths in newborn females, which may be possibly related to lower birth weights in this gender. Mortality in adult saiga was negligible, as indicated by a very low density of carcasses in the area, affecting exclusively female individuals. Reproductive disorders were the leading cause of mortality. The absence of dead adult males is an expected finding in a normal calving year, given the ecology of the species. Therefore, any evidence of adult male mortality in this period demands a further detailed investigation.

The unavailability of complementary diagnostic exams, such as microbiological analysis of animal tissue samples, was a constraint of this study. It is also recommended that, in future investigations of saiga calving aggregations using a similar sampling method, transects be repeated by the observers or, ideally, that a second team begins transects a few days later. This would enable a better quantification of the mortality, as it would pick up any additional carcasses after the passage of the first team. Despite these limitations, the data reported in this study provides a general picture of the normal dynamics of birth and mortality in the calving season of saiga antelopes, which is useful to improve the monitoring of future calving aggregations, as well as the early detection of events leading to unexpected mortality.

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Necropsy Report Form

NAME OF PROSECTOR:

SPECIES: *Saiga t. tatarica* ASSIGNED ID:

WEIGHT (if applicable): SEX: AGE GROUP: C S-A A

ESTIMATED TIME OF DEATH: DATE OF NECROPSY:

LOCATION OF CARCASS: DD° MM′ SS″

ENVIRONMENTAL CONDITIONS: (rain / wind, wet / dry, sunny / cloudy, temperature)

GENERAL OBSERVATIONS: (e.g. topography, vegetation character, water presence, any previous signs of sickness, position of the body, tracks, signs of struggling, signs of predation, other animals, etc.)

EXTERNAL EXAMINATION: (body condition, mucous membranes, skin, hair coat, feet, parasites, discharges etc.)

MUSCULOSKELETAL SYSTEM: (muscles, bones, joints)

BODY CAVITIES:

Fat stores: 1 (poor: almost no fat)

2 (average: fair amount of fat present, but kidneys clearly visible)

3 (good: plentiful fat, completely obscuring kidneys)

Abnormal fluids (blood, exudate, transudate)

Neonates: assess hydration (tissue moistness)

HEMOLYMPHATIC: (Spleen, lymph nodes, thymus)

RESPIRATORY SYSTEM: (Nasal cavity, larynx, trachea, lungs, lymph nodes)
Neonates: determine if breathing occurred (do the lungs float in formalin?)

CARDIOVASCULAR SYSTEM: (Heart, pericardium, great vessels)
Pericardial effusion? (Blood, exudate, transudate)

DIGESTIVE SYSTEM: (Mouth, teeth, oesophagus, stomachs, intestines, liver, pancreas, mesenteric lymph nodes).
Neonates: is milk present in stomach and/or small intestine?

URINARY SYSTEM: (Kidneys, ureters, urinary bladder, urethra)

REPRODUCTIVE SYSTEM: (Testis/ovary, uterus, vagina, penis, prepuce, prostate, mammary glands, placenta)

ENDOCRINE SYSTEM: (Adrenal, thyroid, parathyroid, pituitary)

NERVOUS SYSTEM: (Brain, spinal cord, peripheral nerves)

SENSORY ORGANS (Eyes, ears)

Other observations or comments: